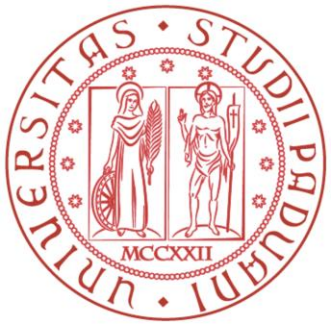
RABBIT DAMS' INFLUENCE IN THE OFFSPRING MORTALITY

Davi Savietto, Matteo Gelain



UNIVERSITÀ DEGLI STUDI DI PADOVA

Dipartimento Agronomia Animali Alimenti Risorse Naturali e Ambiente

Dipartimento di Medicina Animale, Produzioni e Salute

CORSO DI LAUREA MAGISTRALE IN

SCIENZE E TECNOLOGIE ANIMALI

RABBIT DAMS' INFLUENCE IN THE OFFSPRING MORTALITY

Relatore:

Ch.ma Prof.ssa Antonella DALLE ZOTTE

Dipartimento di Medicina Animale, Produzioni e Salute

Correlatore:

Dott. Davi SAVIETTO

GenPhySE, Université de Toulouse, INRA, INPT, ENVT, Castanet Tolosan, France

Laureando

Matteo GELAIN

Matricola n. 1130715

ANNO ACCADEMICO 2016-2017

I. ABSTRACT

Post-weaning mortality is an important drawback in rabbit farming. Nowadays it is mainly related to digestive disorders and, to avoid the economic losses, the sector is highly dependent on the use of antibiotics, the latter consisting in a problem of public concerns. With the aim of reducing the dependency of antibiotics use and, at the same time, of reducing the post-weaning mortality, several strategies have been developed like the use of fibrous feeds and feed restriction practices. More recently some authors started to argue that the pre-weaning period may influence the survival ability later in life. Although some works describes the influence of the maternal microbiota on the post-weaning mortality, none have described to which extend differences in the maternal microbiota may influence the post-weaning mortality of offspring. To this end an experiment have been designed to identify if the maternal microbiota differs among females having big losses of offspring in the post-weaning period from those having few. But to this end the first step is to correctly distinguish between the "good" from "bad" mothers, and this is the main purpose of the present work. Based in the results of a Spanish work, that described that 22 % of mothers were responsible for about 50 % of the post-weaning mortality, we aimed to describe if this pattern is repeatable in other rabbit populations. If yes, under which conditions and what other pre-weaning factors may influence it? After analysing the historical data of 2197 females of the INRA strain 1777 (between 2004 and 2015) it was concluded that the pattern described in Spain is repeatable in other rabbit populations. Among the pre-weaning factors influencing the results, it was observed a negative influence of the number of offspring milked and weaned on the post-weaning survival. It was also observed that our results are highly influenced by the presence of digestive disorders. When digestive disorders were present, the pattern described by the Spanish team resulted repeatable, but when the main mortality was due to respiratory problem, more females (33 %) contributed to the post-weaning mortality.

II. RIASSUNTO

La mortalità post svezzamento è attualmente un problema notevole nell'allevamento del coniglio. Oggigiorno è principalmente legato a problemi digestivi, e per evitare notevoli perdite economiche, il settore è ancora molto dipendente dall'uso di antibiotici. Con l'obiettivo di ridurre la dipendenza dall'uso di antibiotici e allo stesso tempo di ridurre la mortalità post svezzamento, sono state messe in atto diverse strategie, come l'utilizzo di una dieta con maggior contenuto di fibra e restrizioni nella somministrazione del mangime. Più recentemente alcuni autori hanno iniziato a discutere sul fatto che ciò che accade nel periodo di lattazione potrebbe influire nella mortalità nel periodo successivo allo svezzamento. Sebbene alcuni lavori abbiano descritto l'influenza del microbiota materno nella mortalità post svezzamento, nessuno ha ancora descritto in che modo il microbiota materno va ad influenzare la mortalità post svezzamento dei conigli. Con questo obiettivo, un lavoro è stato avviato per capire se il microbiota materno si diversifica tra le fattrici che hanno molte perdite tra i conigli allattati nel post svezzamento e quelle che non ne hanno, o ne hanno poche. A tale scopo, il primo step riguarda la possibilità di distinguere correttamente tra fattrici "migliori" e "peggiori", e questo è stato l'obiettivo principale di questo lavoro di tesi. Partendo da una spagnola, la quale ha osservato che il 22 % delle madri peggiori è risultata responsabile di circa il 50 % della mortalità post svezzamento, nel presente contributo sperimentale si è cercato di capire se questo pattern è ripetibile in altre popolazioni di conigli. Se si, sotto quali condizioni e quali altri fattori intervengono durante la lattazione? Dopo aver analizzato dati raccolti tra il 2004 e il 2015 di 2197 fattrici di linea INRA 1777, possiamo concludere che il pattern osservato in Spagna è riproducibile anche in altre popolazioni. Tra i fattori che durante la lattazione hanno influenzato i risultati, è stata osservata una correlazione negativa nel numero dei conigli allattati e la sopravvivenza post svezzamento. I risultati ottenuti sono stati fortemente influenzati dalla presenza di problemi di tipo digestivo. Quando la causa di mortalità è stata prevalentemente una disfunzione intestinale, il pattern descritto dal team spagnolo è stato replicato, ma quando la principale causa di mortalità era di tipo respiratorio, un maggior numero di fattrici (33 %) ha contribuito al 50 % della mortalità post svezzamento.

Summary

1. INTRODUCTION

1.1 *Rabbits meat production in the World and in Europe*

Unlike chicken, cattle and pork meat, which has a worldwide distribution, rabbit meat production is restricted to few countries. In the world, the average rabbit meat production is 1.56 million tons per year. China is the first producer (762.6 thousand tons in 2014) followed by Italy (269 k tons/year), North Korea (151.9 k tons/year), Egypt (64.9 k tons/year), and other European countries like Spain (63.8 k tons/year) and France (53.3 k tons/year). See **Figure 1.1.1**

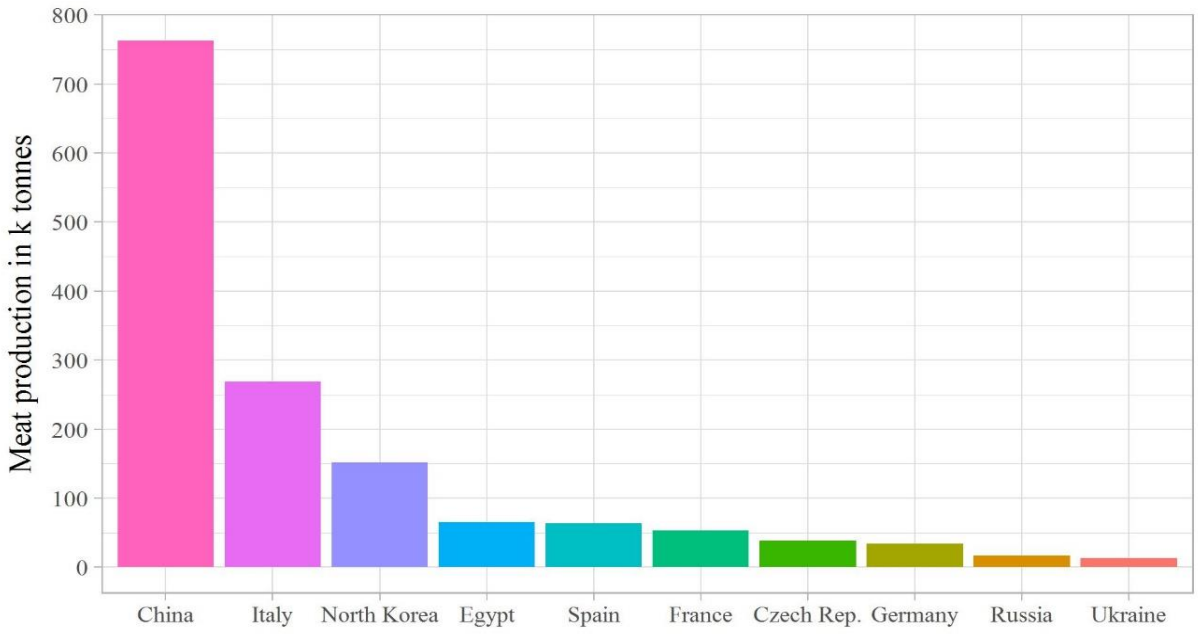


Figure 1.1.1 Top 10 rabbit meat producers in 2015 - (adapted from www.fao.org/faostat)

Together, the European countries produced 522.74 k tons in 2014, and according to FAO (2015), among the main producers in the continent, France is the only country that exports its production. The Italian and the Spanish productions are integrally destined for the national market.

For what concerned the number of animals farmed, there is a total of 769 million rabbits and hares distributed worldwide (census from 2014); numbers that have been growing linearly since 2000 (**Figure 1.1.2**). From these, 107 million are located in Europe being 68.4% located in Italy and 7.7% in France. The growing of worldwide production is principally due to the Asian developing countries, as China, North Korea, Uzbekistan,

Kazakhstan and Tajikistan. The Europe production instead is more constant during these years, with little variations (**Figure 1.1.3**), being the pattern principally explained by the Italian production.

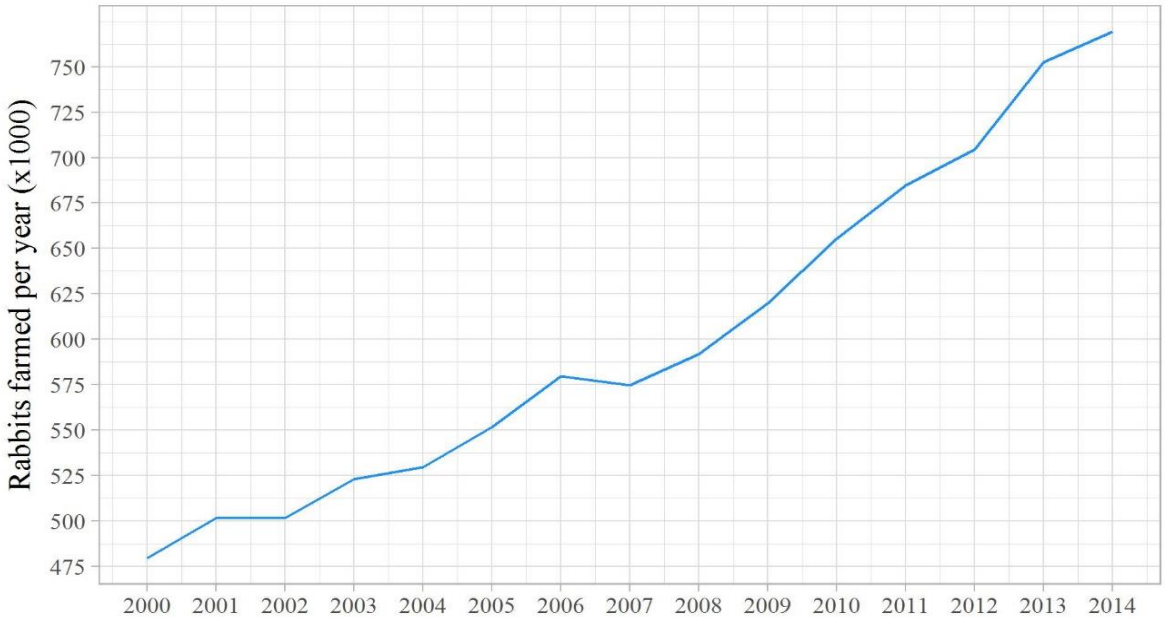


Figure 1.1.2 Number of rabbits and hares farmed in the world between 2000 and 2014 - (adapted from www.fao.org/faostat)

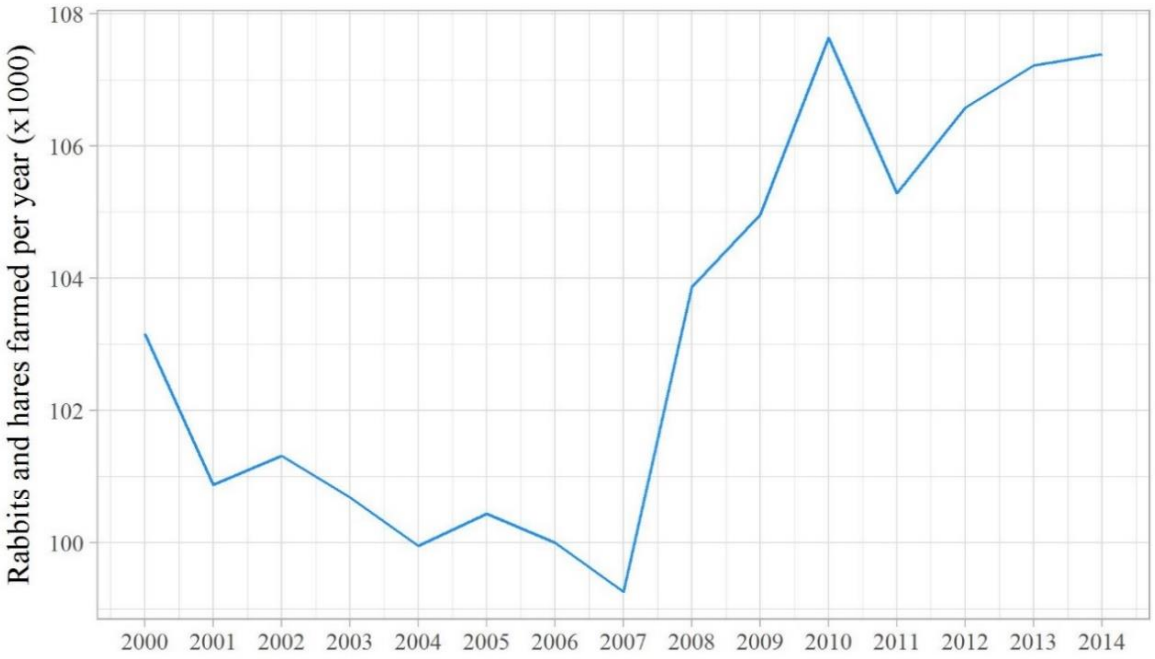


Figure 1.1.3 Number of rabbits and hares farmed in Europe between 2000 and 2014 – (adapted from www.fao.org/faostat)

The variation in the number observed in the last 15 years was very limited in Europe (**Figure 1.1.3**) and in Italy (**Figure 1.1.4**): from 2000 to 2007 there has been a small decrease. From 2007 up to now, rabbit population is grown. France however shows a strong reduction in the number of rabbits and hares farmed: between 2000 and 2014, the number of live animals farmed declined almost 40% (**Figure 1.1.5**).

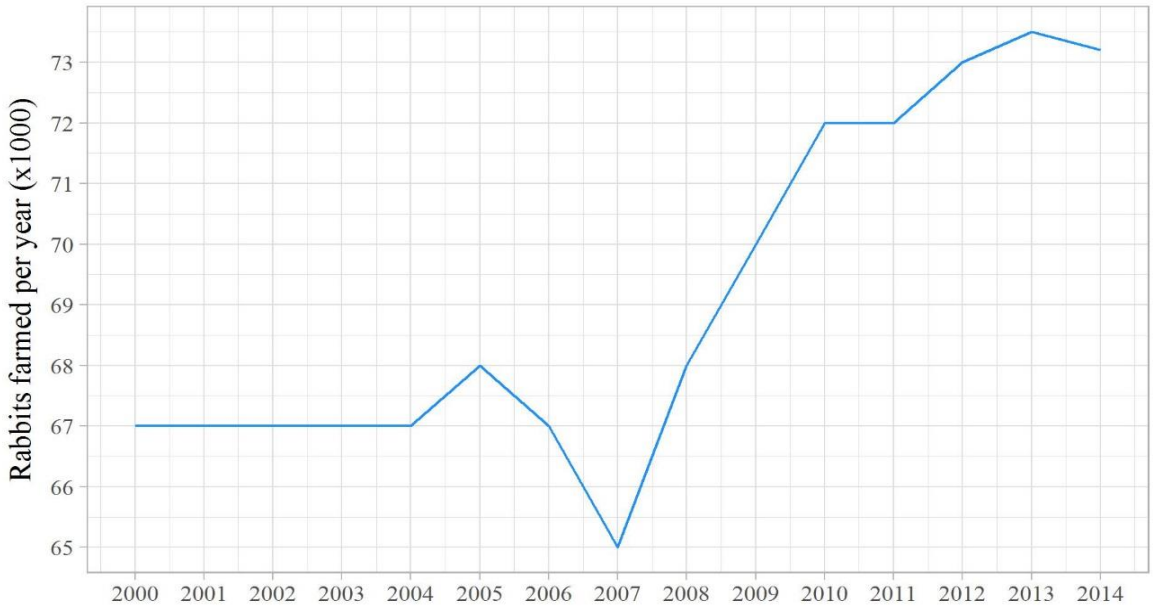


Figure 1.1.4 Number of rabbits and hares farmed in Italy between 2000 and 2014 - (adapted from www.fao.org/faostat)

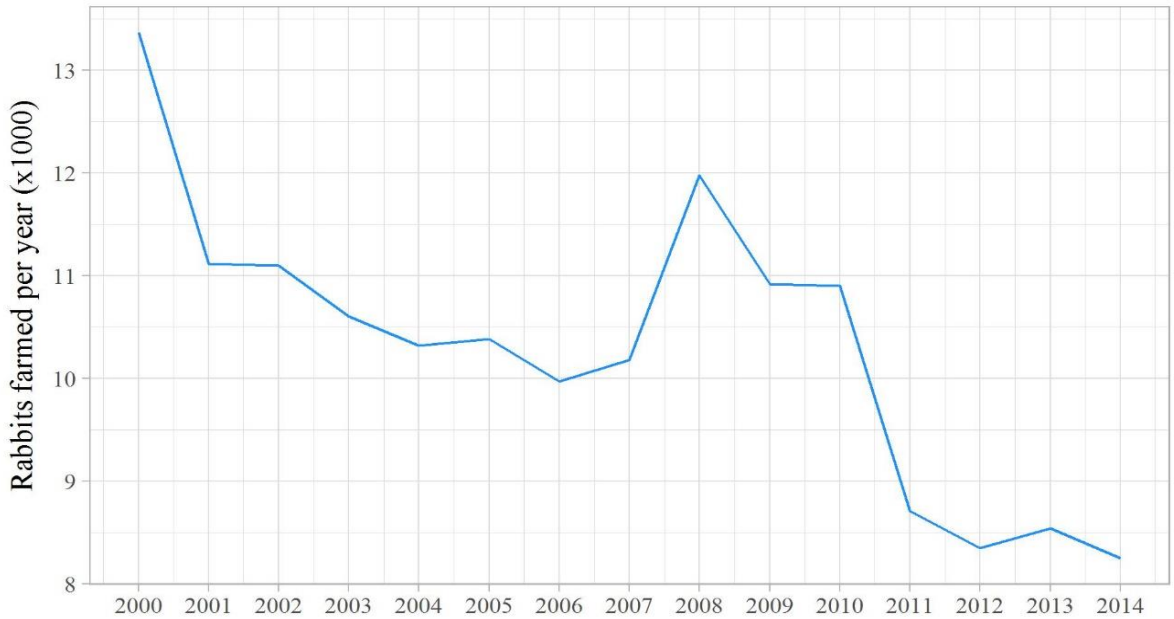


Figure 1.1.5 Number of rabbits and hares farmed in France between 2000 and 2014 - (adapted from www.fao.org/faostat)

1.2 Rabbit intensive farming, its interest and main problems

One of the main interest in raising rabbits is its high prolificacy and the capacity to convert forages and other low valuable feedstuffs in protein of high value, being able to convert 20% of their protein intake into meat (Dalle Zotte, 2014). Rabbits have also a short generational interval compared to others domestic species. A female can conceive just a few hours after giving birth, and when they are managed in a semi-intensive production cycle (i.e. when matted 11 days post-partum), she could have, on average, 6 litters in a single year. If we considering an average litter size of 10 offspring, a mortality rate between birth and market of approximatively 27% and a carcass weight of 1.6 kg, a single female may produce 70.1 kg of meat a year.

Offspring mortality is a big issue. Between birth and weaning, the average mortality rate varies from 6 to 10% and from weaning to market age the values can be as high as 11-12% (Xiccato et al., 2007). During lactation, the main causes of death are human errors in the care of the nest, low maternal ability and diarrhea. During the growth period, digestive troubles are the main cause of morbidity and death. In fact, Peeters et al. (1988) already observed that the main losses are associated with diarrhea, whose etiology is multifactorial. Since 1991, the rabbit production in Europe is particularly affected by the Epizootic Rabbit Enteropathy (ERE). When present, this high contagious illness could kill up to 30-40% of all exposed animals in just few days (Licois et al., 2005, www.anci-aia.org).

1.3 Short description of rabbit digestive physiology

A particularity of the rabbit digestive physiology is its caecum. With respect to other herbivores, this organ is well developed and contains a microbial population that is responsible to ferment the fibrous parts of the diet that the rabbit cannot digest (Gidenne et al. 2010). The caecum also plays an important role in the coprophagous behavior of the rabbit. Together with the proximal colon, this organ is responsible to the formation and the separation of the soft feces and the hard feces. The soft feces, rich in vitamins and proteins of microbial origin (Gidenne and Lebas, 2006), are re-ingested directly from the anus while the hard feces, composed of non-digestible fibrous constituents and little more, are excreted. The process of ingesting the soft feces is called coprophagy, and it is an evolutionary strategy to overcome the poor-quality protein or low vitamin contents of the raw material that the rabbit encounters in its natural environment.

1.4 Mother influence

1.4.1 Nest environment

Rabbit offspring (**Figure 1.4.1.1**), like other altricial mammals, are completely dependent on their mothers to survive due to its low level of independence in thermoregulatory, sensory, locomotor and nutritional function (Coureaud et al., 2010). Since rabbit females spend a reduced time (between 3 to 5 min per day) with their progeny, nest quality, litter size, offspring birth weight and milk intake are essential elements conditioning rabbit offspring survival.

At the onset of parturition, rabbit females start to prepare a nest by shaping straws (or the available material) into it and plucking their own body hair (**Figure 1.4.1.1**). This behaviour, which is regulated by a combination of specific hormones (as oestradiol, progesterone, testosterone and prolactin), are essential to the offspring survival (Gonzalez-Mariscal et al., 1996; Gonzalez-Mariscal, 2001). Females give birth in the nest and, as each young emerges, the mother cleans the newborn, eats the placenta, and nurses the newborn before leaving the nest (Melo and Gonzalez-Mariscal, 2003).

The nest structure and the presence of littermates are essential for an adequate thermoregulation. In fact, the offspring has to rely on the thermoregulation capacity of the litter huddle and the insulating efficiency of the nest structure. (Hoy and Selzer, 2002; Gonzalez-Mariscal et al., 2000; Zarrow et al., 1965). When the environmental temperature is lower than 30-35°C, offspring searches for and maintain body contact with their littermates (**Figure 1.4.1.2**) (Hull and Hull, 1982). For this reason, when the environmental temperature is low, new-borns of small litters are at higher risk of death.

Figure 1.4.1.1 Rabbit offspring of 1 day old in their nest made by mother's hair and wood shavings. The rabbits are completely furless and blind (personal photos).

Figure 1.4.1.2 Rabbit offspring of 9 days old in their nest made by mother's hair and wood shavings. Note how the individuals are close together even when they already have the body completely covered by fur. The room temperature when the photo was taken was 21°C (personal photo).

1.4.2 Birth and lactation period

Females give birth after 31 days of gestation. Rabbit lines selected for prolificacy usually have big litters which can contain up to 20 offspring (**Figure 1.4.2.1**), but in general, the average of the specie is 7 to 9 young (Manning et al. 1994). Since females have only 4 or 5 pairs of nipples, in the case of high prolific females there are almost always more offspring than nipples available. One behavior that may create a similar opportunity for the newborns to suckle is the fact that they switch nipples every 10-20 secs (Hudson and Distel,

1983).

Another important aspect is the number and the time of visits to the nest. Females normally visits the nest once a day (Hoy and Selzer, 2002) and for no more than 3 to 5 min (Zarrow et al., 1965; González-Mariscal, 2007). In this short time, newborns locate the nipples in a few seconds and drink up to a quarter of their body weight in a single nursing (Lincoln 1974; Hudson and Distel 1983, 1995). The single daily visit to the nest in wild environment, for Hudson and Distel (1982), is a behavior that rabbit females evolved to reducing the predation risks (**Figure 1.4.2.1**).

During the first two weeks of lactation offspring weight gain is strongly correlated with the milk intake (Drummond et al., 2000). Furthermore, milk ingestion has been shown to be positively correlated with birth weight and growth rate (Hardman et al. 1970; Drummond et al., 2000). After that, the total amount of milk produced daily from each mother is significantly related to the litter size, and to the average of body weight of the offspring. Another factor that can influence the growth of the litter, is the age of the mother: in wild environment, growth is lowest in litters of 1 year old mothers, increase in litters of 2 and 3-years-old mothers, to decreases again in litter of older mothers (>3 years); a pattern related to the female's social rank (Rodel et al., 2008). In commercial farms, where the females are isolated, and the animals have no direct contact to each-other and therefore no hierarchy is stablished, Quevedo et al., (2003) described higher weight gain of offspring from the 4^{th} and 5^{th} births with respect to offspring from the 1^{st} and the 2^{nd} litters. These results indicate an age effect on the females' milk yield.

For what concern the mortality related to the milk ingestion, there are two important factor to note: the first one is related to the share of milk amongst the littermates in the nest. If the litter is very large, the milk share for each offspring could be insufficient (Mendl, 1988; Hudson and Trillmich 2007). The second factor is related to development degree of offspring at weaning. Quevedo et al. (2003) argued that faster growth rates during lactation may increase the risks related to the weaning period, because the passage from one feed supply to another is more abrupt (offspring with high milk availability start to feed solid food later or in smaller quantities with respect to offspring with lower milk availability). This fact also impacts the post-weaning period. In fact, Pascual et al., (2001) noticed that rabbits who ingested less milk showed a faster growth rate in the post-weaning (growing) period

Figure 1.4.2.1 Rabbit offspring of 1 days old in their nest made by mother's hair and wood shavings. Note how mother take care of the nest, trying to exclude the death rabbit from the others. Note also that the female does not remain in the nest with the offspring, also if the passage is available (personal photos).

1.4.3 The establishment of gastrointestinal microbiota in the newborn

The microbiota in the gastrointestinal tract plays a fundamental role, particularly in herbivores. For them it is a source of high value proteins. Regarding the health and the immune response, Sekirov and Finaly (2009) explained that microbiota located in the gut contribute to improve the resistance to infection through their involvement in the development of the host immune system and provision of colonization resistance.

At birth, the gastrointestinal tract of a young mammal is almost sterile and it is gradually colonized through exposure of microorganisms. The bacterial communities of digestive tract of the newborn rabbit is characterized by a few very abundant species that change during the life to contain more, but less diverse, species which is normally observed in adults (Curtis and Slaon, 2004; Berg, 1996). There is also a huge fluctuation in the rabbit gastrointestinal bacterial community composition from the lactation period (between days 7 to 28 of life) until 49 days old (Combes et al., 2011), a period, which in the rabbit, is particularly important for the maturation of the immune system in response to the exposure to the microbial community present in the gut (Fortun-Lamothe and Boullier, 2009).

In this aspect, the nursing mother microbiota plays a fundamental role on the microbial colonization of newborns. In fact, some authors (Combes et al., 2014, Mackie, 1999) described that caecal colonization happening during the lactation, prevailed over the microbial community observed around birth. And so, for the gut microbiota colonization, it is more important the role of the milking mother, than the role of the biologic mother (Abecia et al., 2007).

Mackie (1999), hypothesized that during lactation, the gut microbial community of the rabbit offspring is significantly influenced by that of the mother. However, the caecal microbiota of the rabbit offspring does not develop significantly until they start to consume solid feed. According to Padilha et al. (1995) it happens around the 3rd week of age. For this reason, the newborn rabbit is more susceptible to several enteric infections (Gidenne 1997, Licois and Gidenne 1999), which can imply mortality rates in this period near 25% (Koehl 1997).

Based in the normal behavior of rabbit females to defecate in their own nest, it has been hypothesized that this behavior may influence the offspring gut microbiota colonization through the contact and/or ingestion of the mother's feces (Hudson et al. 1996; Kovacs et al. 2006 – **Figure 1.4.3.1**). In addition, it was shown that the caecal contents of a young rabbit double its weight between the third and the fifth week of life (Lebas and Laplace 1972), in a period that corresponds to the beginning of ingestion of solid material (mother's feed and faeces). Furthermore, the stabilization period of gut microbiota continues in the post-weaning, when the production and the intake of the soft faeces (caecotrophes) starts (de Blas and Wiseman 1998).

Figure 1.4.3.1 Rabbit offspring of 9 days old in their nest made by mother's hair and wood shavings. Note the presence of hard feces of the mother. At this age offspring is going to start to open their eyes. (personal photo).

As Combes et al., (2014) described, if the offspring have a great amount of hard faeces (from their mothers) available in the nest, they eat a bigger amount of it. These authors also

shown that it stimulated the bacterial colonization, changed the bacterial community, and improved the health index (measured as mortality plus morbidity) of rabbit offspring up to 80 days old. Contrary, the mortality rate among rabbit offspring that had no access to their mother's hard feces (i.e. the nest was cleaned and changed every day) was higher. At this point, it is wide accepted that the microbiota of the newborn rabbit is determined by the microbiota inherited from the nursing mother and by the different material ingested at young ages.

The bacterial community is also influenced by the diet. The diet is a source of nutrients to the animal and to its gut bacterial community and therefore, the absence of a key subtract that is essential to the growth of a particular bacterial community limits its presence. For example, when mice were fed on low fiber diet during several generations, their gut bacterial diversity were reduced (Sonnenburg et al., 2016). Another interesting fact observed by Sonnenburg et al. (2016) is the fact that the recovery of the gut diversity related to a change from a diet that favored a low bacterial diversity to a diet favoring a high microbial diversity was related to the amount of diversity presented in the host (a factor influenced by the generation; **Figure 1.4.3.2** - adapted from Sonnenburg et al., 2016).

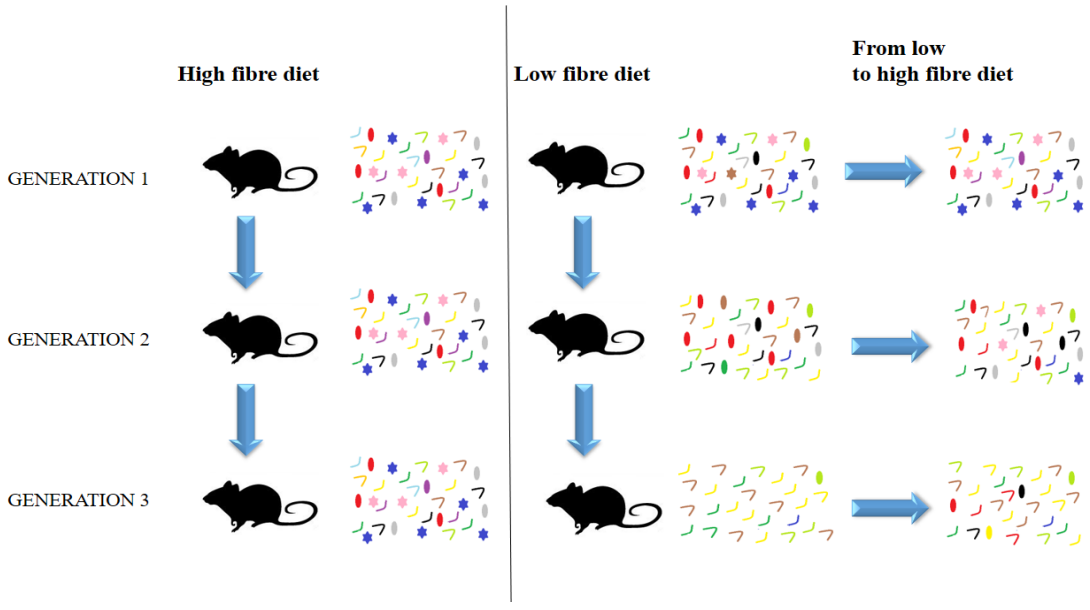


Figure 1.4.3.2 Loss of diversity. Adapted from Sonnenburg et al. (2016). These authors found that mice fed a low-fibre diet had a lower species diversity in their gut microbiota than mice fed a high-fiber diet. In first-generation mice, most (but not all) of this diversity was recoverable when mice on the low-fiber diet were switched to a high-fiber diet. However, the authors found that diversity loss was greater in each subsequent generation maintained on a low-fiber diet, and that the degree of recovery also decreased, implying extinction of some microbial species.

In the case of rabbits the intergenerational transmission of the microbiota happens in the

nest through the mother's hard feces. If the results from mice are transposable to the rabbits, it logically implies that offspring from mother with low fiber diet should have a less diverse microbiota with respect to offspring from a mother fed with a high fiber feed. A result that has been observed by Martens (2016). The study of Sonnenburg et al. (2016) exemplifies that that changing the diet is not a solution to re-establish the initial microbiota diversity observed in the first generations, the solution would be to put the newborns in contact with nursing females (or their feces) from a group of female rabbits fed on a high fiber diet through several generations.

In the situation of immunity immaturity, the weaning entails important variations in the environment, as new cage, new littermates, separation from the mother, new feed, that can change the ability of immunity response of the animal (Gallois al., 2005), that could be insufficient to cover all the animal needs. However, if the animals are prepared to the weaning in advance, the risks related to this event may be reduced, influencing the results (in terms of growth and mortality risks) observed in the post-weaning period.

1.4.4 Post weaning

In commercial farms, rabbits are usually fed *Ad libitum* after weaning. This period begins with the separation from the mother at about 30-35 days of age and ends around 45-50 days of age. This is the most critical period for the success of breeding and for the health of animals, which are more susceptible to the digestive disease. The main causes of this susceptibility are the rapid development of the digestive tract, the colonization and development of a new microbial flora in the caecum, the beginning of the cecotrophy mechanism and, finally, the stress caused by the separation from the mother.

The one of the most important risk factor is the feeding factors. Feed composition and the alimentation program have great influence on the appearance of digestive troubles. In what concern its composition, the level and the type of fiber (de Blas and at., 1999; Gidenne, 2000) combined to the level of protein (Carabaño and at., 2002) are the most important constituents affecting the normal function of the rabbit gastrointestinal tract. Indeed, for Carabaño et al. (2005), the nutrition is considered one of the most significant factor influencing the normal functioning of the gut and the animal health. Feed also plays an indirect role in the health process due to the interaction between the gut membrane and the microorganisms, which together form one of the major barrier of the animal (Carabaño et al., 2005). For Carabaño et al., (2005) the change of feed that occurs around weaning may negatively affect the barrier characteristics of the small intestine. Contrarily to what is

observed in the small intestine, a precocious weaning seems to be favorable to the large intestine, once the bacterial colonization that favors the diversification of the gut microbiota and the development of the immunity system start earlier (Carabaño et al., 2005; Gallois et al., 2005).

After weaning at least two different diets are used: one for the post-weaning period and one for the fattening-period. As Xiccato and Trocino (2008) explained, the main differences between these two diets principally refers to the starch and neutral detergent fiber contents. The quantity of protein has a little variation, compared to the other two components (an example of the chemical constituents of these diets can be seeing in **Table 1.4.4.1**). High level of starch in the diet is normally associated to the emergence of digestive disorders during weaning and post weaning period (de Blas and Gidenne, 2010). The protein level in the diet should be about 15-16% of crude protein. A lack or a surplus of protein can favor digestive disease and increase mortality rate. An outcome related to the protein imbalance which modifies the intestinal microbiota and fermentative capacity of the caecum (Carabaño et al. 2008; 2009). In relation to the fiber. Low levels of insoluble fiber reduce the intestinal transit speed, extending the permanence of the feed in the intestine. This increase the protein fermentation because bacteria have more time for the fermentation. This process causes an ammoniacal nitrogen and pH increasing and then it promotes intestinal dysbiosis (Gidenne, 1996).

The fibre, in particular when the soluble fibre replaced the starch, in diet with a similar amount of acid detergent fibre, improves the health status of the rabbit (Xiccato et al. 2008), being important to the correct development of the gut microbiota. It also has the function to improve the stimulation of the immune response and protection against pathogens, contributing thus to the host resistance to infection through the provision of colonization resistance (Sekirov and Finlay, 2009). Until this point it is clear that the pre-weaning factors described above may influence the post- weaning mortality.

Table 1.4.4.1 Chemical composition of three reference diets for growing rabbits (adapted from Xiccato and Trocino, 2008)

	Diet		
Chemical composition	**Weaning**	**Fattening**	**Finishing**
Dry matter (DM; %)	88.5	88.7	88.5
Crude protein (% of DM)	16.0	15.5	14.9
Neutral detergent fiber (% of DM)	35.0	33.4	31.7
Starch (% of DM)	8.5	14.0	18.5

1.4.5 Mortality influenced by bad mothers

The post-weaning mortality of the young rabbits seems to be influenced by the mothers. For this reason, Quevedo et al., (2003) utilized 496 litters from 150 mother to understand if the mothers could have any influence in the post-mortality.

In their work, Quevedo et al. (2003) observed that the 22% of mothers were responsible for about 50% of the total post-weaning mortality. This mean that some mothers actually influence the mortality through their parity order, the litter size, the feed received during lactation and /or the weaning weight. However, others factors, like the maternal microbiota (passed thought hard feces, excreted in the nest during the lactation) may influence the offspring's survival ability in the post-weaning period.

In the light of these results, an experiment was designed to identify differences in the maternal microbiota that may influence the offspring survival ability during the post-weaning period. The first phase of this project consists in identify if the pattern observed by Quevedo et al. (2003) is robust enough to be used to distinguished between mothers contributing a lot from mother contributing a few to the post-weaning mortality. The second phase is to collect fecal samples (hard feces) from the mothers during lactation, and analyze them. The aim is to see if the mothers with high litter mortality in the post-weaning are characterized by the presence of some particular bacterial strains, and looking for microbiota differences between "bad" and "good" mothers, considering "bad", the ones belonging to the 22% of females that show higher mortality. Today there are very few information for what concern the mother influence in the post weaning deaths, and with these works we would like to find more information about it.

2. PROJECT HYPOTESIS

Are there some females that contributes more than other to the post-weaning mortality? If yes, why? Is it only a casual factor or there are other factors behind this influence? If the mother influences the mortality rate of its litter, which are the factor that can affect their litter health? Is the number of offspring dead linked to what happened in the pre-weaning period? Could be the female's hard feces that are normally excreted in the nest, and thus the microbiota that the mother passes to its litter influence the post-weaning survival ability?

Complementary to what I am looking for with the historical data of rabbits at the experimental station of INRA Occitanie - Toulouse, samplings of hard feces are being collected from all females of a long term-experiment. The long term aim of this project is to characterize the microbiota of three groups of females: the ones having a high influence on the post-weaning survival ability of their offspring, those having a low influence and females with no mortality.

3. THE AIM OF THE PRESENT EXPERIMENT

The main aim consists to test the hypothesis that some mothers contribute more than others to the overall post-weaning mortality, or reject it and conclude that post-weaning mortality is not directly influenced by the nursing female. However, if the hypothesis that some mothers that contributes more than others to the post weaning mortality will be confirmed, the following aim will be to identify the pre-weaning conditions that litters were exposed which could influence the results.

4. MATERIAL AND METHODS

4.1 Data Set

Historical data (from June 2004 to March 2015) of 2 240 rabbit females, farmed at Pôle Expérimental Cunicole de Toulouse (PECTOUL) of the Institute National de la Recherche Agronomique (INRA), were used. All animals belonged to the genetic strain INRA1777; a strain selected for litter size at birth. The data set contained information on 14 generations of selection: from generation 35 to 48 and the number of females per generation is given in **Table 4.1.1**.

Table 4.1.1. Number (*n*) of females per generation of selection for litter size at birth.

	Generation of selection for litter size at birth													
	35	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**
n	157	178	189	169	119	78	174	96	172	143	215	162	143	158

Due to the particularities of the selection program, only the data of the 4th (*n* = 1 061) or 5th (*n* = 1 179) parturition of females were available within each generation. Moreover, due to another experimental protocol, a short number of females belonging to the generation 45 (41 females of 215) were moved to another farm. To avoid all the unknown factors affecting the performance of these females and their progeny housed in this foreign facility, the data of these 41 females were not considered in the final analysis. The data set made only by animals farmed in Langlade farm was composed by 2 197 females (less 2 females excluded for no information).

4.2 Farming practice at the selection nucleus

Females were first inseminated at 136 days old (19.5 weeks old) being re-inseminated every 11 days' post-partum, resulting in a programed birth-to-birth interval of 42 days. Litters were weaned at 32 ± 3 days old and fattened rabbits were sent to the market at 73 ± 3 days old. After birth, the average number of offspring assigned to each female was 9, but some variation through the selection program occurred (the range is between 7 and 13, as shown in **Figure 4.2.1**).

The different animal categories (young females, reproducing females, and growing rabbits) were fed with commercial diets especially designed to meet their particular nutritional

requirements. Water was freely available and depending on the age and physiological state of the animals feed intake may be restricted to avoid over-fattening (future breeding females) or digestive disorders (growing rabbits). No antibiotics were administered in Langlade farm.

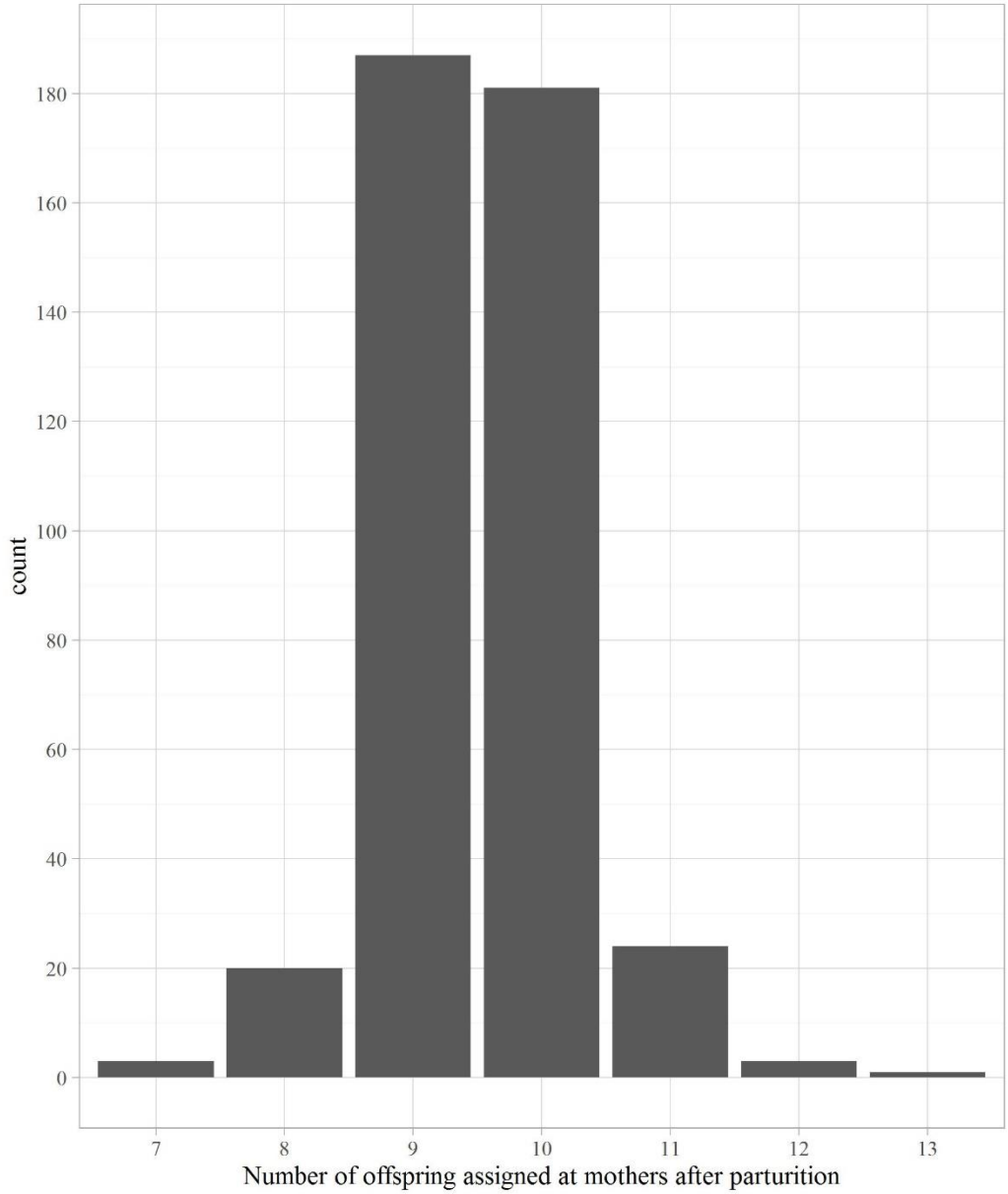


Figure 4.2.1 Histogram with the count of mothers with the same number of offspring milked.

For the rabbit feeding only one feed were used, starting at 21 days old. Until 21 days old they were fed exclusively on mother's milk.

Females were kept in the farm to a maximum of 8 parturition when the new generation takes place (i.e. all females of generation *n*-1 are culled to make space to females of generation *n*). Eliminations occurs due to diseases or low productivity. In the current data set, a total of 317

females were eliminated due to diseases, 450 were found death, 1 357 were culled, and 109 were classified as being alive (7 females with no information).

Selection took place in a single building subdivided into several rooms, and in the case of the line INRA1777, a total of four rooms (called: A, B, C, and D) were used in the period between June 2004 and March 2015. Each time a new generation took place in one of the four rooms and therefore two rooms were never used at the same time. **Table 4.2.1** summarize the generations, the number of females and the number of females contributing to at least one offspring to the overall offspring mortality in the post-weaning period according to the housing room. Note that only generation 48 were housed at room D. In this room (and generation 48), 44.3 % of the females contributed to the post-weaning mortality while in the other rooms (and generations) the value was always below 20.0 %.

Table 4.2.1. Generations, number of females and number of females contributing to the post-weaning mortality housed at rooms A, B, C and D.

Room	Generations	Females (*n*)	Females contributing to the post-weaning mortality (*n*)	Offspring lost in post-weaning
A	35, 37, 39, 41, 43, 45	985	169 (17.2 %)	282
B	36, 38, 40, 42, 44	749	140 (18.7 %)	242
C	46, 47	305	40 (13.1 %)	89
D	48	158	70 (44.3 %)	233

Females gave birth to a total of 22 837 viable offspring. Due to the cross-fostering practice (litters set to 9 offspring, on average), a total of 1 938 offspring were culled, resulting in a total of 20 899 offspring. From these, 3 205 offspring were cross-fostered being nursed by another female, referred as the milking mother. Only 18 520 of the 20 899 offspring raised survived to weaning and 17 674 reached the market age. Offspring mortality during lactation was 11.38 %, during fattening 4.57 %, and 15.43 % in total (22.61 % if we consider the culled offspring in the calculation).

4.3 Cleaning the data set and setting new variables

For the objective of this study, we focused on litters of females (milking mothers) having at least one offspring death in the post-weaning period (between 35 and 62 days old). Although information on the biological mother were available, we focus on the effect of the litter where the offspring were raised (i.e. its milking mother). All these choices affected the effective number of litters to be analyzed, that was reduced from 2 197 to 419.

On the remaining 419 litters, the influence of the generation of selection (from 35 to 48), the housing room (A, B, C and D), the number of offspring born alive (10.41 ± 3.20), the number of offspring milked (9.52 ± 0.76), the number of offspring weaned (8.62 ± 1.26) and the number of death through lactation (0.89 ± 1.23) on the post-weaning mortality were studied.

Contribution of the milking mother (i.e. litter) to the overall mortality observed in the post-weaning period was computed as the number of dead offspring divided by the total number of offspring death in this period (n = 846). This new variable allowed the computation of the accumulated mortality.

The last variable set up was an arbitrary classification of females according to the number of offspring she "lost" in the post-weaning period. Three classes (low, medium and high) were established and the values of each class are present in **Table 4.3.1**.

Table 4.3.1. Number of death offspring in the post-weaning period and number of females (litters) at each arbitrary class for post-weaning mortality (low, medium, high).

Class	**Low**	**Medium**	**High**
Number of death offspring	1	2 or 3	≥ 4 (max = 8)
Number of females (litters)	241	115	63

4.4 Analysis strategy

Data was analyzed with the free software R (R Core Team, 2016). Figures were drawn using the package "*ggplot2*" (Wickham, 2009) and the statistical analysis using the internal package "*stats*". Several linear regressions were performed to study the pre-weaning factors affecting the post-weaning mortality. The first model [1] related the post-weaning mortality (*PWM*) to the number of offspring born alive (*BA*) as:

$$PWM_i = \mu + \beta \cdot BA_i + e_i \quad \textbf{[1]}$$

The second model [2] related the *PWM* with the number of milked offspring (*NM*) as:

$$PWM_i = \mu + \beta \cdot NM_i + e_i \quad \textbf{[2]}$$

The third and fourth models considered the influence of the offspring weaned [3], dead offspring during lactation [4] in a similar way of models [1] and [2]. It is worth nothing that generation of selection, the housing room, the mother birth season and the offspring birth season were effects that cannot be take into account separately once all females of a single generation were housed in a specific room at a given time. Therefore, the results of the proposed models are just indicative of a group of combined effects. In this sense, no conclusions on the main effects of these variables on the post-weaning mortality could be directly performed.

Data were analyzed following the animals' subdivisions: the first analysis was performed with the females with at least one dead offspring during the post weaning period, neglecting all the housing effect and other external factors (419 females). The second analysis was performed with animals farmed in the same room. The third one was performed with animals farmed before and after 2008, when a pathogenic strain of *E. coli* was identified in the farm (see **Figure 4.3.1** for a better understanding of the analysis steps). There were also a generation and year/season effect, but due to a lack of data, we were not able to disentangle all these factors.

In the analyses, we also considered the exit reason of the dead rabbits in the post-weaning period: intestinal infection, respiratory disease or other.

After 2008 the pathogenic strain 0103 of *E. coli* was identified in the farm. From this year, the post-weaning mortality raised from 2.1 % to 6.3%. For this reason, data was further sub-divided in two sets before and after the entrance of *E. coli – 0103* in the farm.

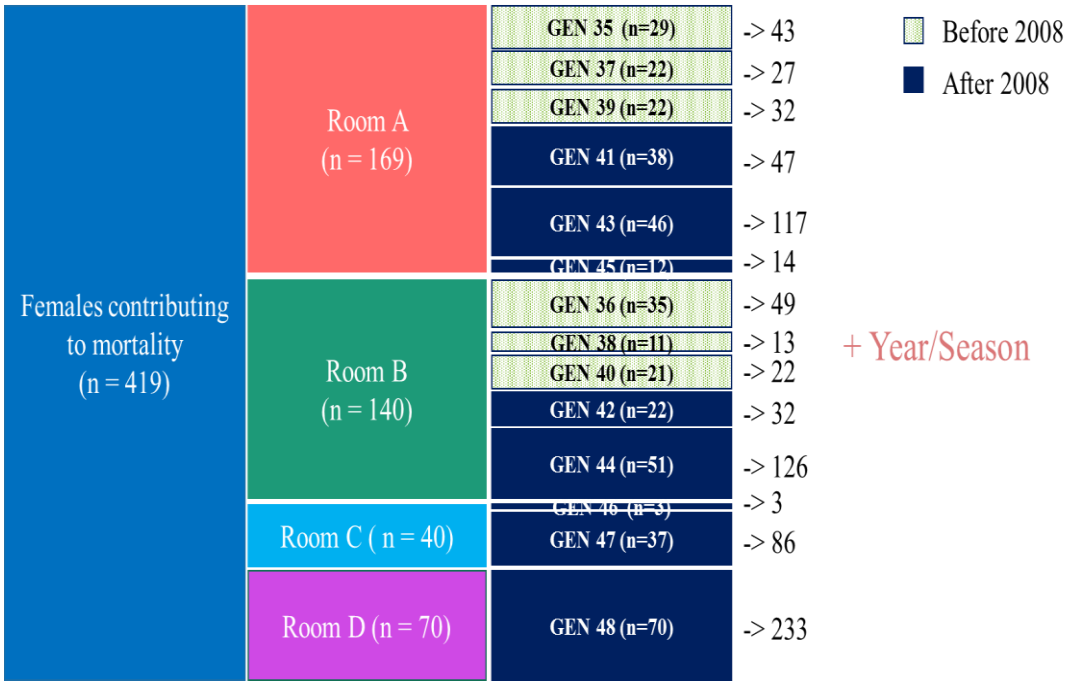


Figure 4.3.1 Number of females with at least one offspring dead according to the housing room and generation of selection. Year season effect is also present, but were not considered in the analysis due to the limited number of animals available in each of these classes. Numbers in parenthesis represents the number of females and the numbers outside the rectangles represents the number of offspring dead in the post-weaning period in each generation of selection. Note the high values of post-weaning mortality for generations 43, 44, 47 and 48 when the herd was exposed to the pathogenic *E. coli* strain *O103*.

5. RESULTS

5.1 Percentage of mothers responsible to the overall post-weaning mortality

First statistical analysis approach was used to identify, among the mother having at least one offspring dead in the post-weaning period, those responsible of a high amount of deaths, as pointed by Quevedo et al. (2003). From our data, we observed that only 22 % of mothers were responsible for ≈ 50 % of the overall post-weaning mortality (423 offspring died from only 93 mothers), and the dead offspring range for females was between 1 and 8 as shown in **Figure 5.1.1.a-b**. The 22 % of worst females had between 3 and 8 dead offspring in post-weaning period.

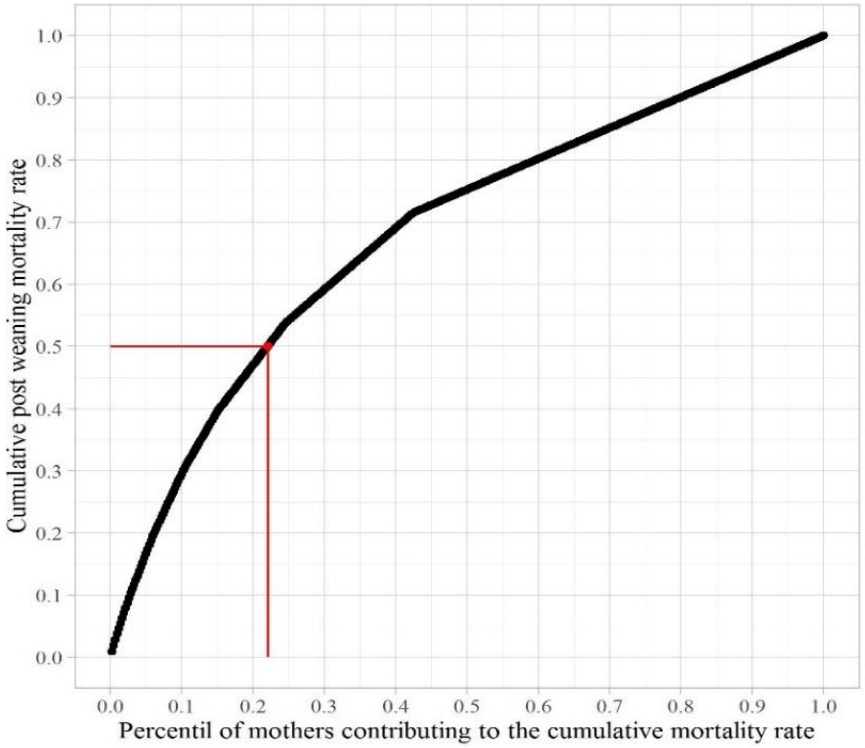


Figure 5.1.1 a. Cumulative percentage of post-weaning mortality rate shown by females ordered in a decreasing ordered for the number of death offspring.

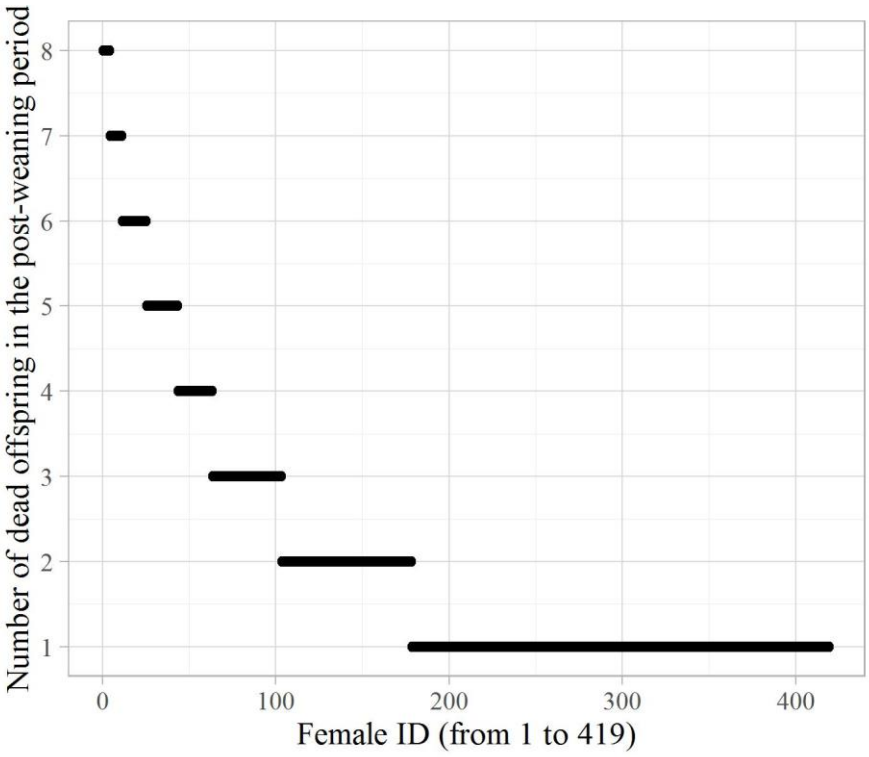


Figure 5.1.1 b. Number of dead offspring in post-weaning period ordered in decreasing order.

From the overall mortality observed in the farm, we had information on its main causes. Looking the rabbit exit reasons in post-weaning period, it is important to underline that digestive infection was the main cause (613 rabbits), followed by respiratory diseases (166) (**Figure 5.1.2**). In particular, a total of 89.3 % of rabbits lost due to digestive disorders were raised by the worst 22 % of mothers (those contributing to the 50 % of the overall post-weaning mortality). Only the 8.8% of them died for a respiratory disease, and the remaining 1.9% for other non-specified reasons. A similar pattern was observed for offspring of the remaining 78 % of the mothers contributing to the post-weaning mortality; the main exiting reasons were digestive (incidence of 55.9 %) followed by respiratory (30.6 %) and other non-specified reasons (13.5 %).

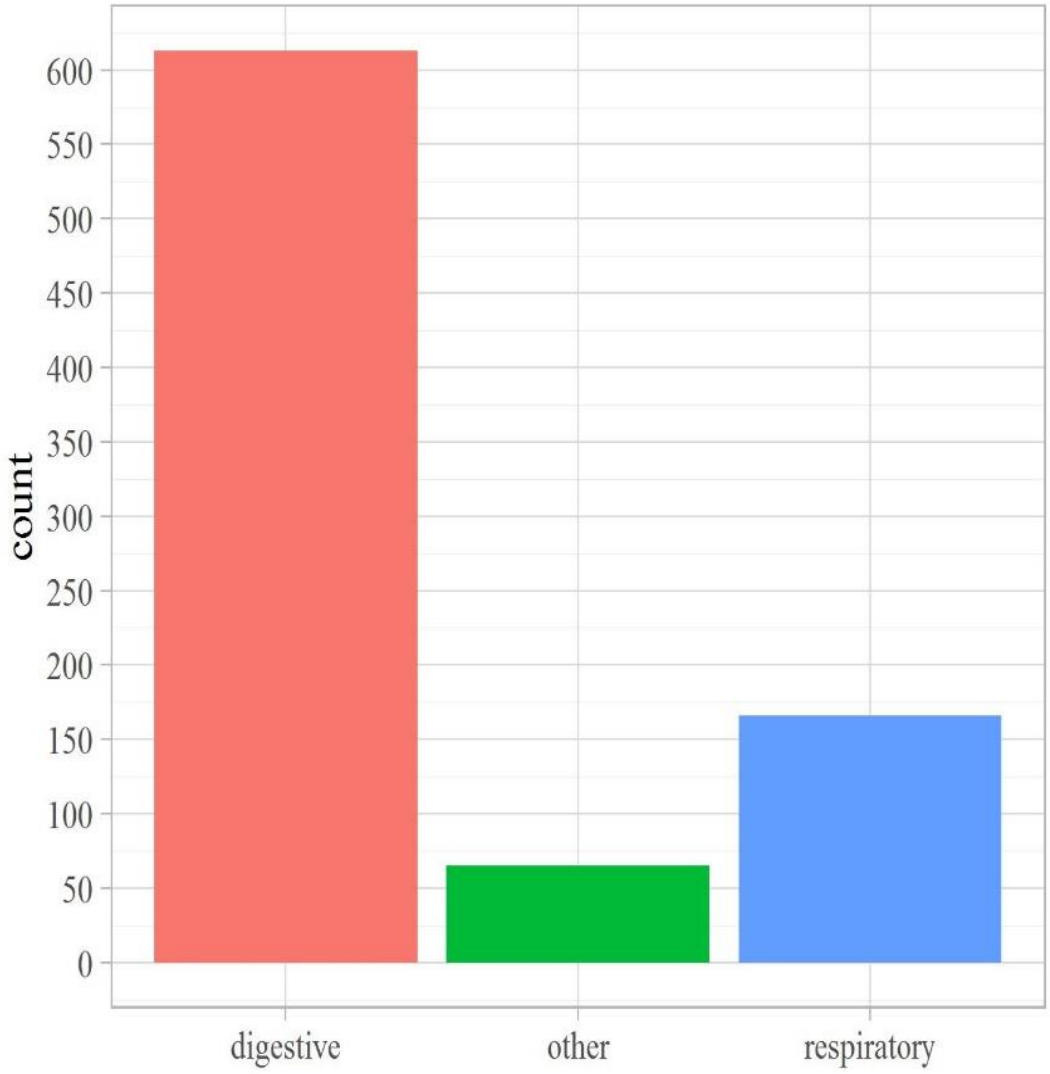


Figure 5.1.2. Main causes of post-weaning offspring mortality (digestive, respiratory and other non-specified reasons).

5.2 Room effect

Accounting for the room effect as a source of variation, the second analysis was made to identify the percentage of females responsible to ≈ 50 % of the post-weaning mortality in a given room.

Independently on the housing room, similar pattern was observed at each one of the four housing rooms. The results were similar to that observed for the whole population; about 25 % of females contributed to ≈ 50 % of the overall post-weaning mortality. Although, we found no relevant differences in the percentage of females contributing to ≈ 50 % of the overall post-weaning mortality among the housing rooms (**Figure 5.2.1**), it is important to

point out that animals of the more recent generations of selection that were housed at rooms C and D were exposed to the pathogenic *E. coli* strain *O103*.

Irrespectively of all other non-controlled factors, we found the same percentage of females contributing to ≈ 50 % of the overall post-weaning mortality within a single room: A = 24.9 % of mothers, B = 24.3 %, C = 27.5 % and D = 25.6 %. Although in the case of room C, the number of females contributing to the post-weaning mortality was low (only 40 animals), we observed a similar pattern with respect to rooms A, B and D.

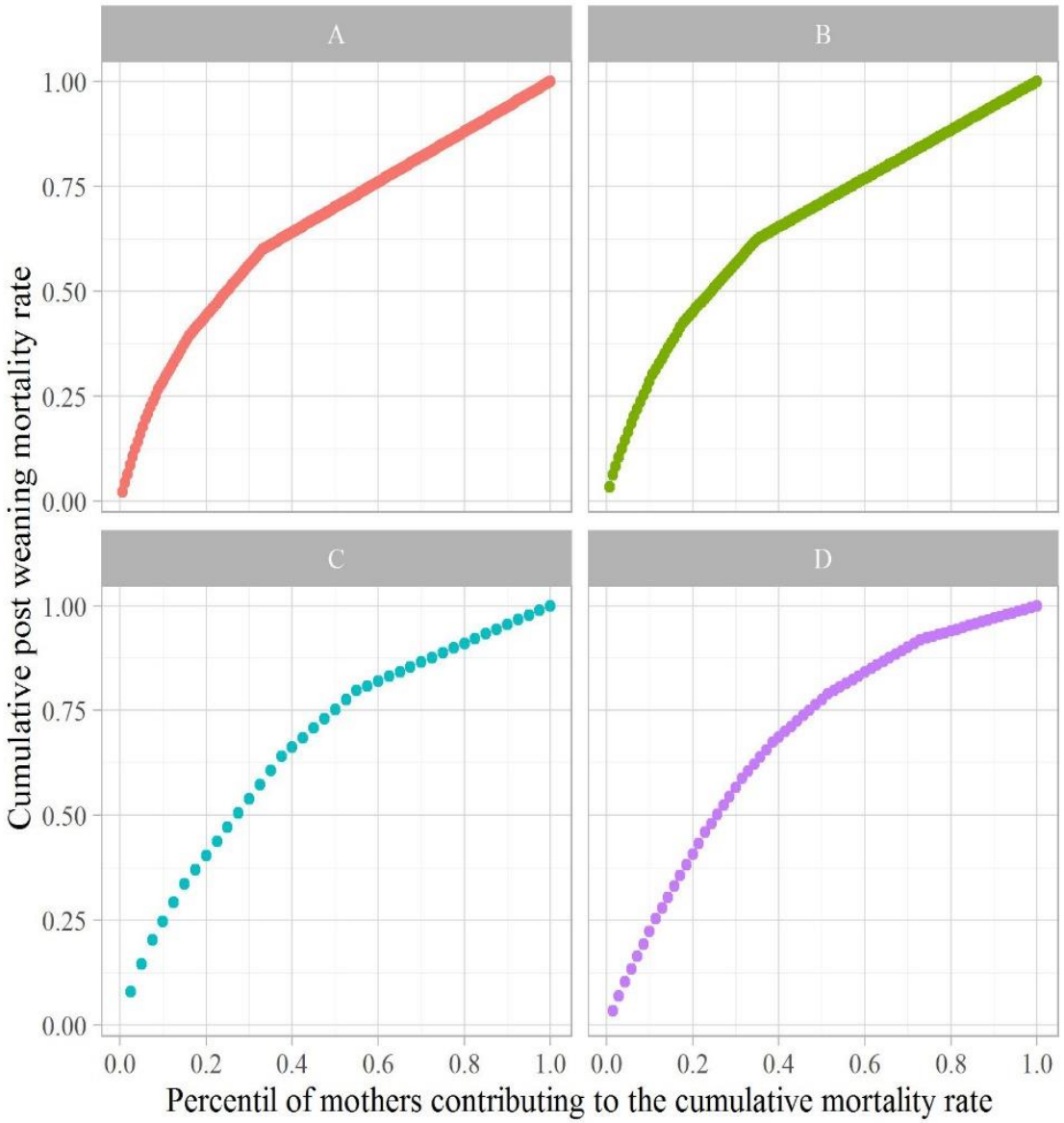


Figure 5.2.1. Cumulative percentage of post weaning mortality rate shown by females in a decreased order for number of death offspring during the post-weaning period for each farming room.

5.3 Exposure to the pathogenic E. coli strain O103

As we mentioned before, from 2008 the farm was affected by pathogenic *E. coli* strain *O103*. Because of the presence of this pathogen entailed a huge increasing on the post-weaning mortality, we sub-divided the data (before and after the entrance of *E. coli*) to quantify at which degree the presence of this bacteria influenced the results.

We observed that before the entrance of this pathogen in the farm (during 2008) ≈ 33 % of females accounted to ≈ 50 % of the overall post-weaning mortality (linked to a short range on the number of offspring dead in the post-weaning from a single litter: range from 1 to 4). After 2008, when the first case of death by *E. coli* started, the percentage of females contributing to ≈ 50 % of the overall post-weaning mortality decreased to ≈ 22 % and the number of death offspring of a single litter reached values as high as 8 deaths (range from 1 to 8). See the distinct shapes of the cumulative mortality curves before and after the entrance of the pathogen in the farm (**Figure 5.3.1a**).

From 2008 some generations shown an incredibly high mortality (especially generations 43, 44 and 48) while the others showed more-or-less a “normal” value (41, 42, 45, 46 and 47). Another interesting point to note is that after the entrance of the *E. coli O103* in the farm, the number of offspring death in a single litter increased (**Figure 5.3.1b**). Therefore, the curvature of the graphic showing data after 2008 (**Figure 5.2.1a** - right) is steeper than the first one (**Figure 5.3.1a** - left).

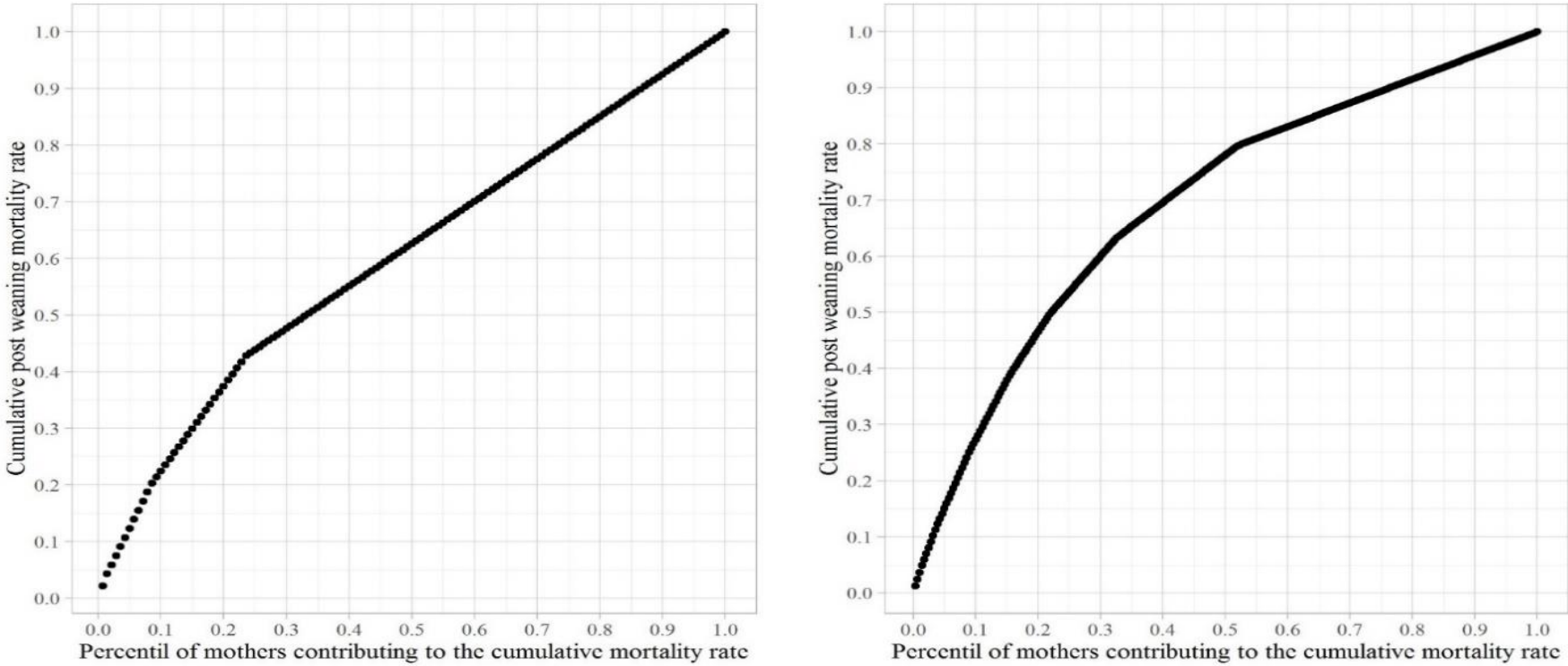


Figure 5.3.1a Cumulative percentage of post-weaning mortality ordered by the contribution of each females (decreasing order) to the overall post-weaning mortality before (left panel) and after (right panel) the entrance of the pathogenic *E. coli* strain *O103* in the farm in 2008.

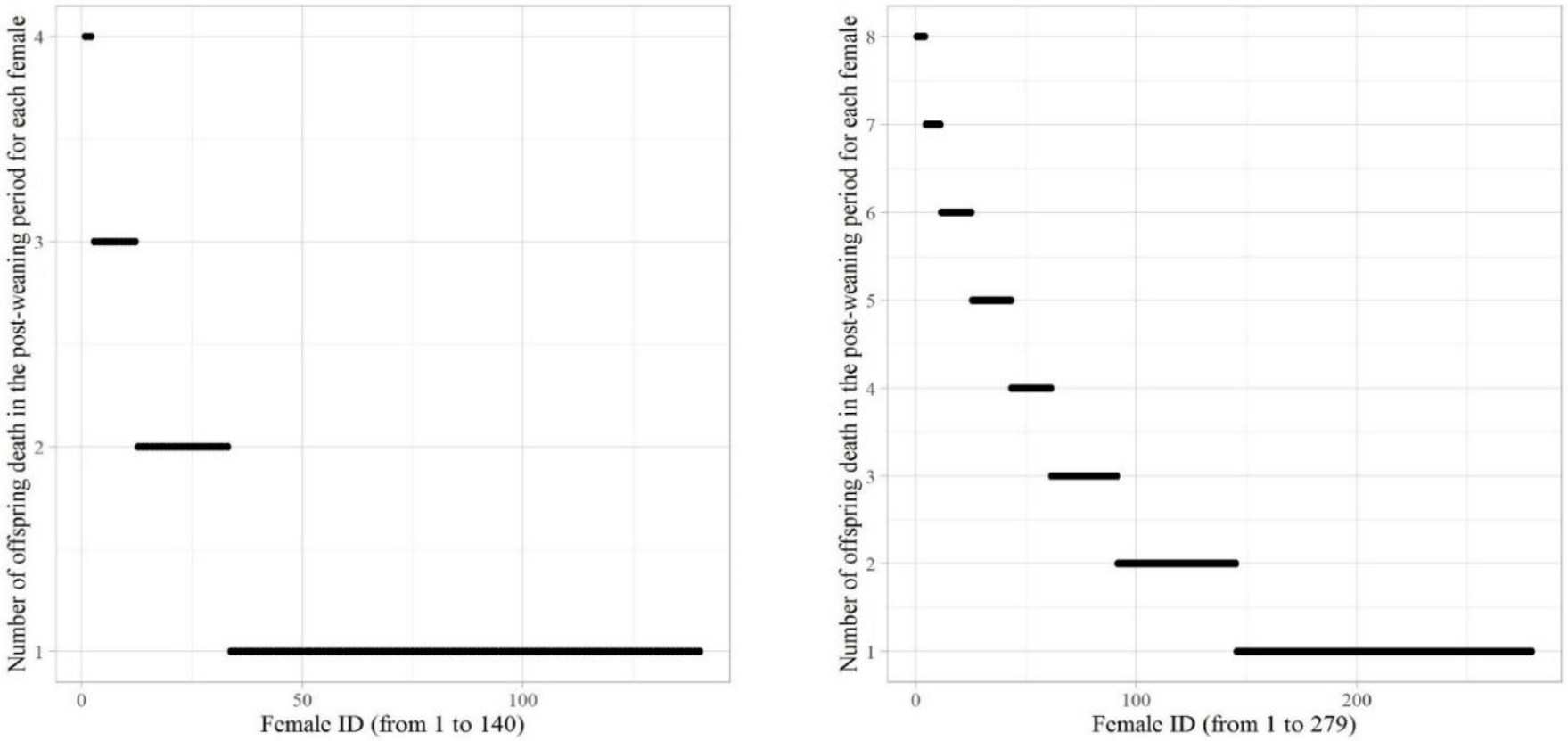


Figure 5.3.1b Number of dead offspring in the post-weaning period for each mother (decreasing order) before (left panel) and after (right panel) the entrance of the pathogenic *E. coli* strain *O103* in the farm (year 2008). Note that the female ID is an arbitrary value (lower ID values represents females with high post-weaning loss).

Main causes of offspring post-weaning mortality before and after *E. coli O103* are in Figure 5.3.2. Before the appearance of this bacteria in the farm, respiratory affections were the main reason of death (or culling); only five offspring were affected by digestive troubles (**Figure 5.3.2** - left). However, after the entrance of *E. coli O103*, most animals died (or were culling) due to digestive disease (**Figure 5.3.2** - right).

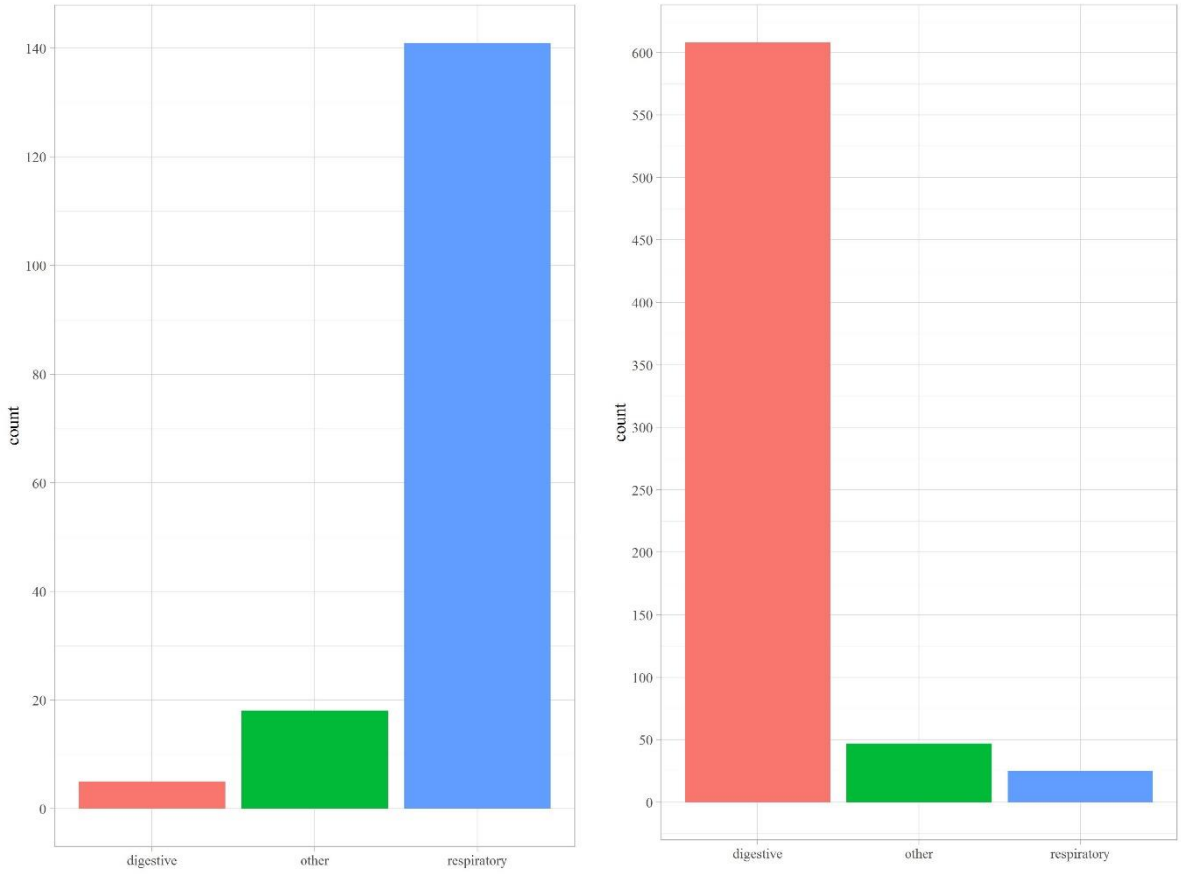


Figure 5.3.2. Main causes of death (or culling) in the post-weaning period. Left panel: before the entrance of the pathogenic *E. coli* strain *O103*. Right panel: after the entrance of this bacteria in the farm.

5.4 Evolution of the post-weaning mortality through generations

In our data, each generation shown a different mortality rate. The percentage of females contributing to the post-weaning mortality also differed. In generation 46 only 0.2 % of mothers had at least one dead offspring while for generation 48 the percentage rose to 15.6 %. In these generations, it was shown the biggest difference in the range of the number of dead offspring per mother: in generation 46 there were 3 mothers with one dead offspring each, instead, in generation 48, the dead offspring range per mother was 1 to 8.

The count of mothers belonging to each class in each generation were presented Figure 5.4.1 (left panel): Low = 1 (red), Medium: $2 \leq x \leq 3$ (green), and High > 3 (blue) dead offspring according to **Table 4.3.1**. The density function of the same pattern was presented in **Figure 5.4.1** (right panel). Note how the number of mothers from High class (more than 3 dead offspring in post-weaning period) increased from generation 43, and how in the last

two generation the number of females from Medium and High classes were incredibly high. It is also important to note that the generations 45 and 46 were characterized by an incredibly low post-weaning mortality.

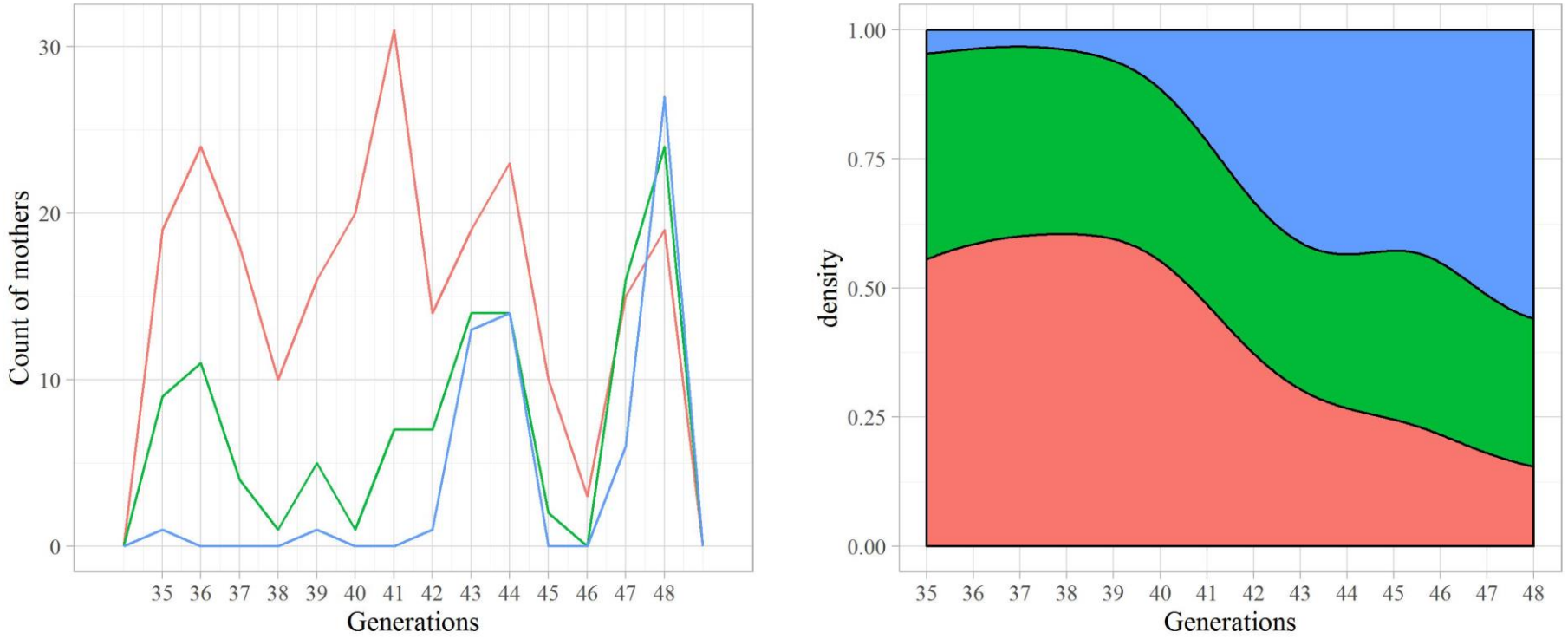


Figure 5.4.1 Females belonging to class "Low" (colored in red; x = 1), "Medium" (green; $2 \leq x \leq 3$) and "High" (blue; $x \geq 4$), according to the classification established on Table 4.3.1. In the left panel, the number of females in each class through generations. In the right panel a density function of females of in each class through generations.

We want to underline that in each generation, before *E. coli*, the mortality rate was low, and the percentage of mothers responsible of 50 % of morality was over 30 % in all generation (excluding the generation 39, in which there was a lower percentage of females responsible of 50 % of mortality, but with very few values available). After 2008 (at generation 41) the percentage of females responsible for 50 % of mortality varied greatly and the number of females with at least one dead offspring increased, as reported in **Table 5.4.1**.

Table 5.4.1 Number of females with at least one dead offspring and the percentage of females that shown ≈ 50 % of mortality in the post-weaning period according to the generation of selection.

	Generation of selection for litter size at birth													
	35	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**
Females (n)	29	35	22	11	22	21	38	22	46	51	12	3	37	70
Percentage	30.7	30.1	39.3	40.9	26.2	47.6	38.2	31.8	23.7	24.5	37.5	50	27.9	25.6

5.5 Other relevant factors: Influence of mothers in the pre-weaning period

After the first result, which is 50 % of offspring died in post-weaning period coming from 22 % of mothers, we deepened to see if there were some aspects in their early life (i.e. through their lactation) that could have influenced their post-weaning survival ability. The effects we studied were the influence of the number of born alive, the number of littermates, the number of weaned offspring and the mortality during lactation on the post-weaning mortality.

Concerning the relationship between the number of born alive and the post-weaning mortality, the coefficient of regression appeared to be not different from zero ($P>0.05$) and therefore the variability on the number of offspring dead in the post-weaning period could not be explained by the number of born alive. In other words, an increase in the number of offspring born alive did not appear to be a factor influencing the post-weaning mortality. However, it is important to note that litters were standardized to 9 or 10 offspring at birth.

The regression equation obtained for the relationship between post-weaning mortality and the number of offspring born alive was:

$$PWM_i = 1.650\ (\pm 0.265) + 0.035\ (\pm 0.024) \cdot BA_i + e_i \qquad [1]$$

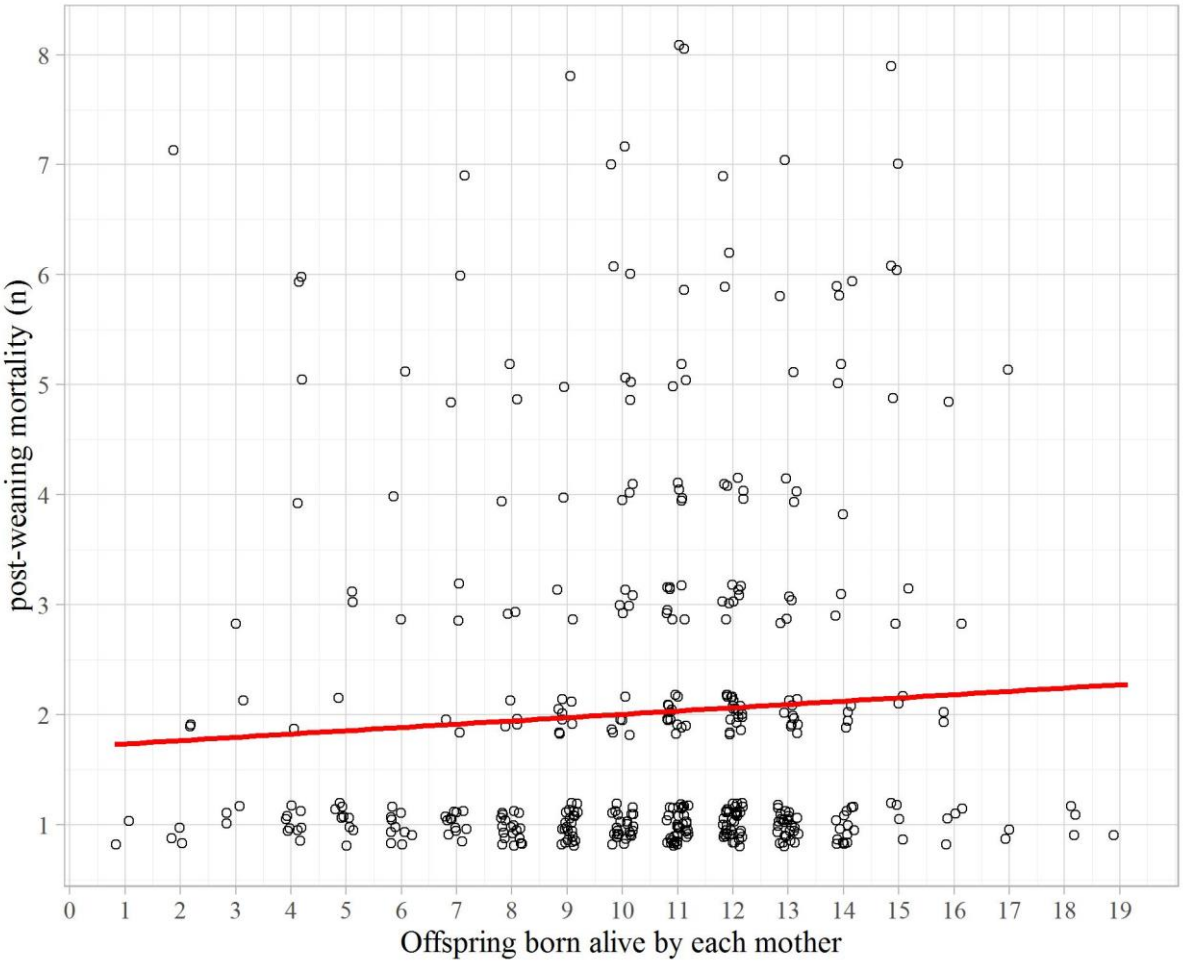


Figure 5.5.1. Linear regression for the number of offspring dead in the post-weaning period on the number of offspring born alive. The points have been jittered close the real one for helping the density visualization.

A different result was found for the relationship between the post-weaning mortality and the number of offspring milked. In this case, the regression coefficient was different from zero ($P<0.05$), and therefore an increment of one offspring milked (OM) represented, on average, an increment of 0.382 offspring dead in the post-weaning period (**Figure 5.5.2**). The regression equation for this relationship was:

$$PWM_i = -1.614\ (\pm 0.967) + 0.382\ (\pm 0.101) \cdot OM_i + e_i \qquad [2]$$

It is important to note that due to the cross-fostering practice, the range of offspring milked varied from 7 to 13, influenced the intercept to be a negative value.

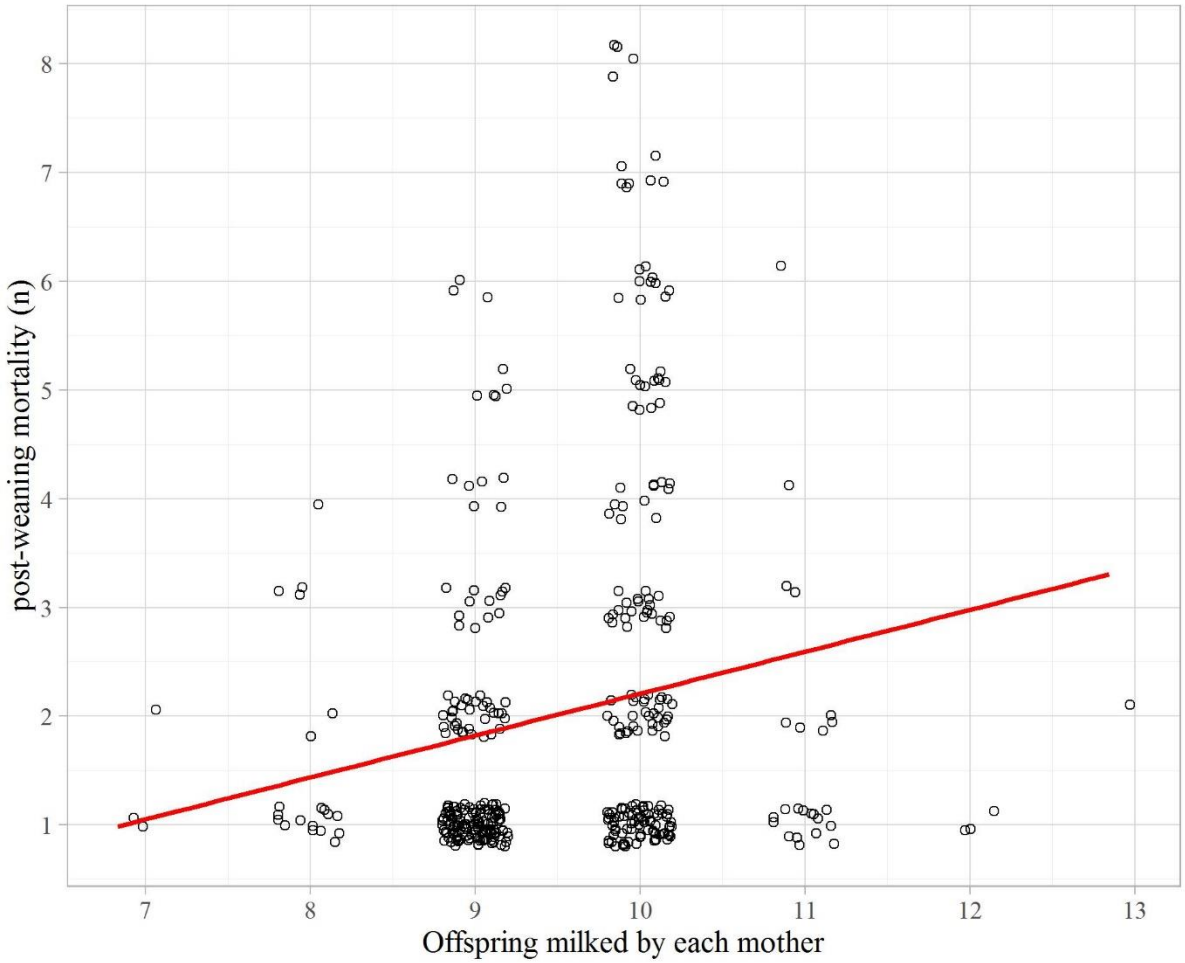


Figure 5.5.2. Linear regression for the number of offspring dead in the post-weaning period on the number of offspring milked. The points have been jittered close the real one for helping the density visualization.

Concerning the influence of the number of offspring weaned (OW) and the post-weaning mortality, the regression equation was:

$$PWM_i = -0.936\ (\pm 0.0.52) + 0.343(\pm 0.060) \cdot OW_i + e_i \qquad [3]$$

The regression coefficient of offspring weaned on post-weaning mortality was different from zero ($P<0.05$). In this sense, an increment of one offspring weaned represented, on average, an increment on the number of offspring dead in the post-weaning period of 0.343 offspring (**Figure 5.5.3**). Again, the fact that the range of offspring weaned varied from four to 11 resulted in a negative intercept.

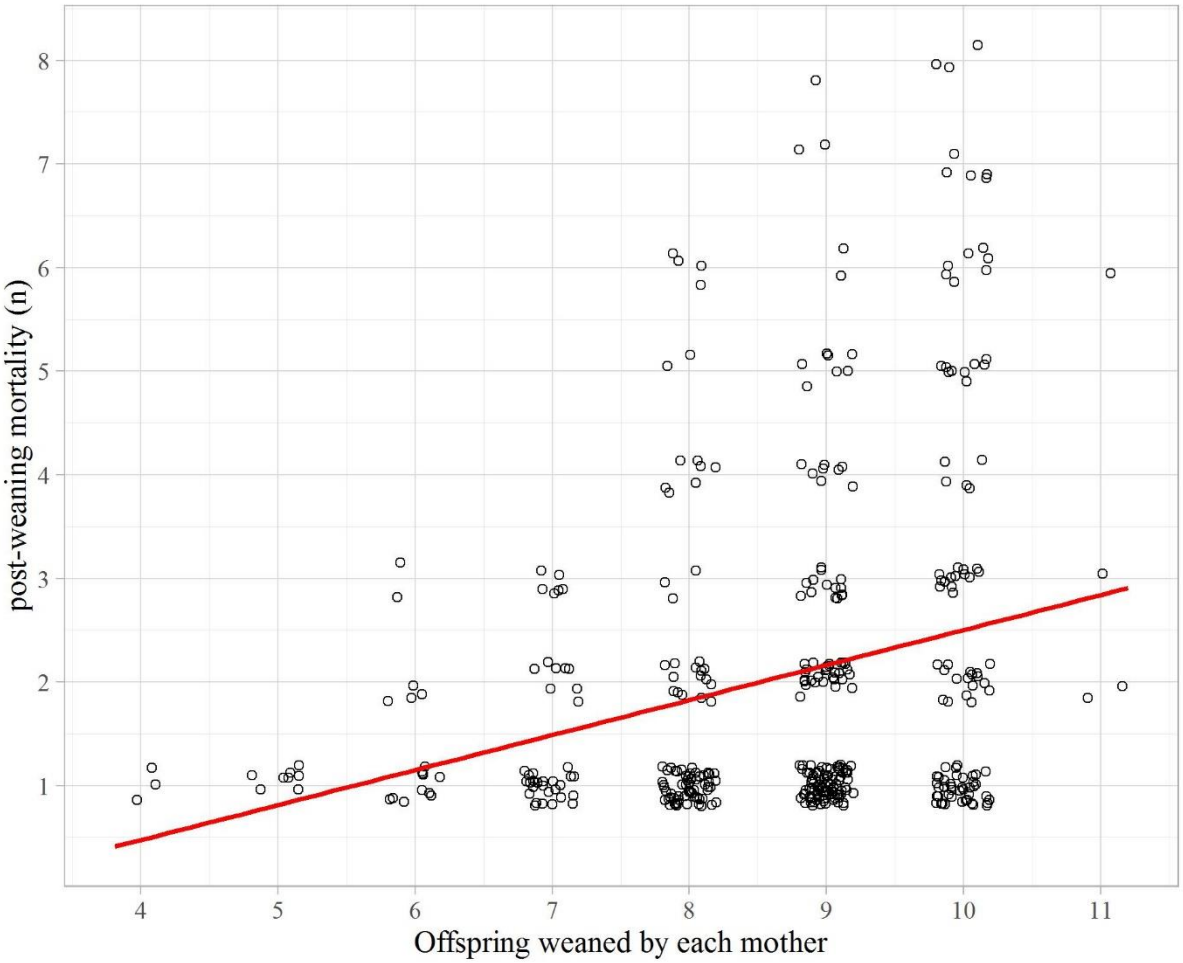


Figure 5.5.3. Linear regression for the number of offspring dead in the post-weaning period on the number of offspring weaned. The points have been jittered close the real one for helping the density visualization.

The final relationship studied was the one between offspring dead in the post-weaning period in function of the offspring dead in the lactation (DOL). A negative relationship ($P<0.05$) was observed according to the linear equation [4]:

$$PWM_i = 2.211\ (\pm 0.095) - 0.214\ (\pm 0.063) \cdot DOL_i + e_i \qquad [4]$$

This equation show that, on average, an increment of one offspring dead during lactation result in a reduction on the post-weaning mortality of 0.214 offspring (**Figure 5.5.4**).

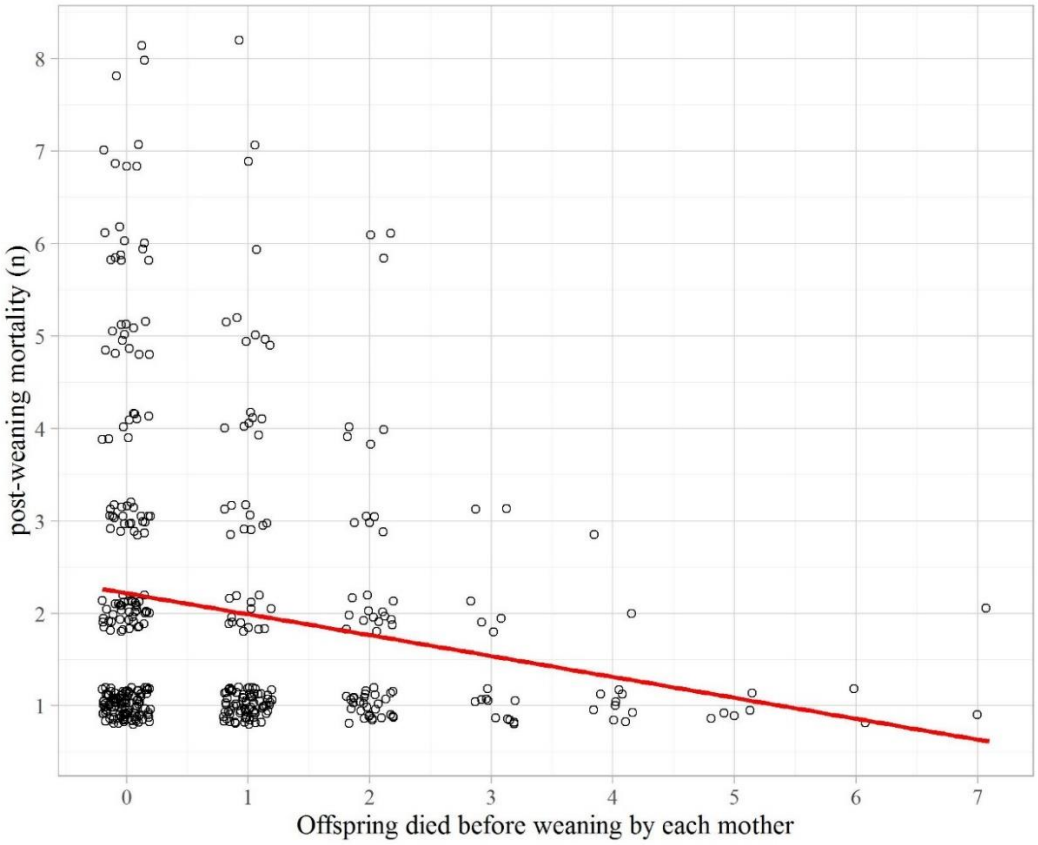


Figure 5.5.4. Linear regression for the number of offspring dead in the post-weaning period on the number of offspring dead during lactation. The points have been jittered close the real one for helping the density visualization.

Knowing that the results could have been influenced by high mortality that occurred after the appearance of the pathogenic *E. coli* strain *O103*, we re-run the test for the relationship between post-weaning mortality and offspring milked, between post-weaning mortality and offspring weaned and between post-weaning mortality and dead offspring during lactation by separating the data set before and after 2008; the year this bacterium appeared in the farm. We choose to not present the same analysis for the relationship between post-weaning mortality and the number of offspring born alive due to the lack of relationship between these two variables in both analysis performed.

The results of this second analysis, show that before *E. coli* there were no significant differences for the post-weaning mortality in function of the number of offspring milked, weaned or dead in pre-weaning (see the left panels of **Figure 5.5.5**, **5.5.6** and **5.5.7**). After the appearance of the *E. coli* strain *O103*, there were no significant differences for the post-weaning mortality in function of the number of offspring milked (regression coefficients equal to zero), instead the number offspring weaned and died in lactation had a regression coefficients different to zero ($P<0.05$); see right panel of **Figures 5.5**.5, **5.5.6** and **5.5.7**

$$PWM_i = 1.132\ (\pm 0.194) + 0.021\ (\pm 0.019) \cdot OM_i + e_i$$

$$PWM_i = -0.601\ (\pm 1.613) + 0.305\ (\pm 0.166) \cdot OM_i + e_i$$

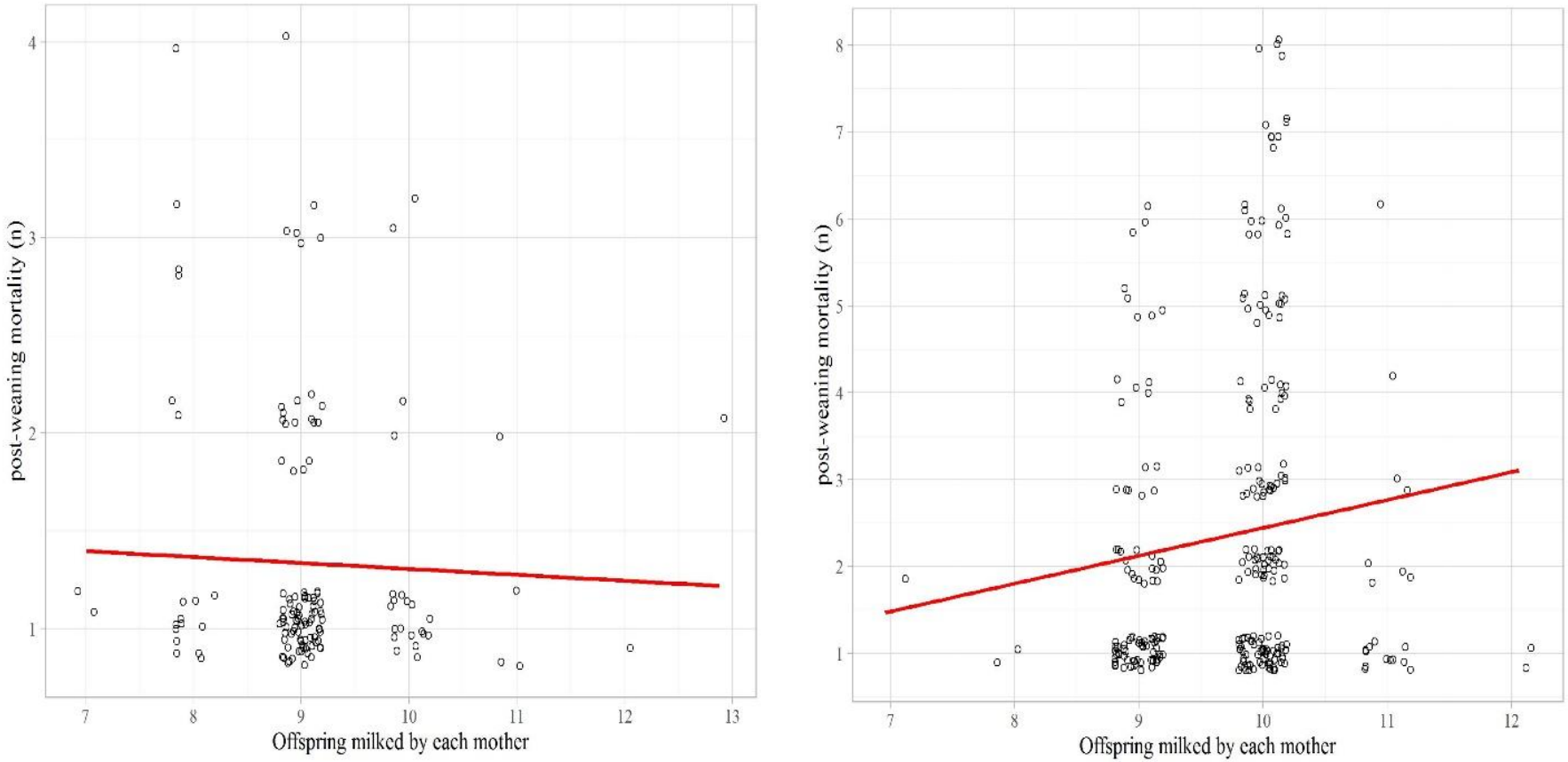


Figure 5.5.5. Linear regression for the number of offspring dead in the post-weaning period on the number of offspring milked. Left panel: before the entrance of the pathogenic *E. coli* strain *O103*. Right panel: after the entrance of this bacteria in the farm. The points have been jittered close the real one for helping the density visualization.

$$PWM_i = 1.444\ (\pm 0.369) - 0.013\ (\pm 0.045) \cdot OW_i + e_i$$

$$PWM_i = -1.219\ (\pm 0.813) + 0.402\ (\pm 0.090) \cdot OW_i + e_i$$

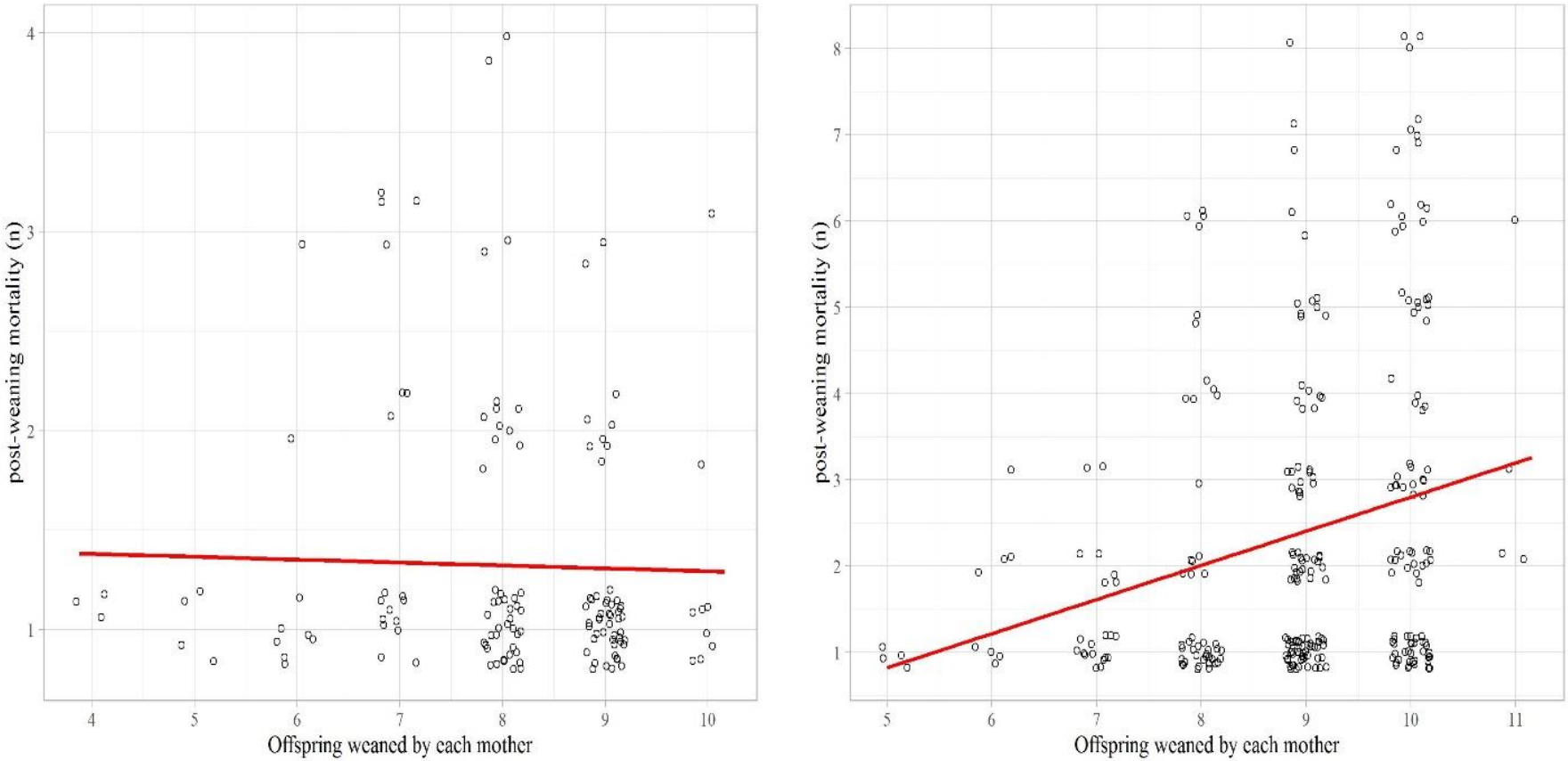


Figure 5.5.6. Linear regression for the number of offspring dead in the post-weaning period on the number of offspring weaned. Left panel: before the entrance of the pathogenic *E. coli* strain *O103*. Right panel: after the entrance of this bacteria in the farm. The points have been jittered close the real one for helping the density visualization.

$$PWM_i = 1.338\ (\pm 0.071) - 0.002\ (\pm 0.040) \cdot DOL_i + e_i$$

$$PWM_i = 2.633\ (\pm 0.132) - 0.330\ (\pm 0.095) \cdot DOL_i + e_i$$

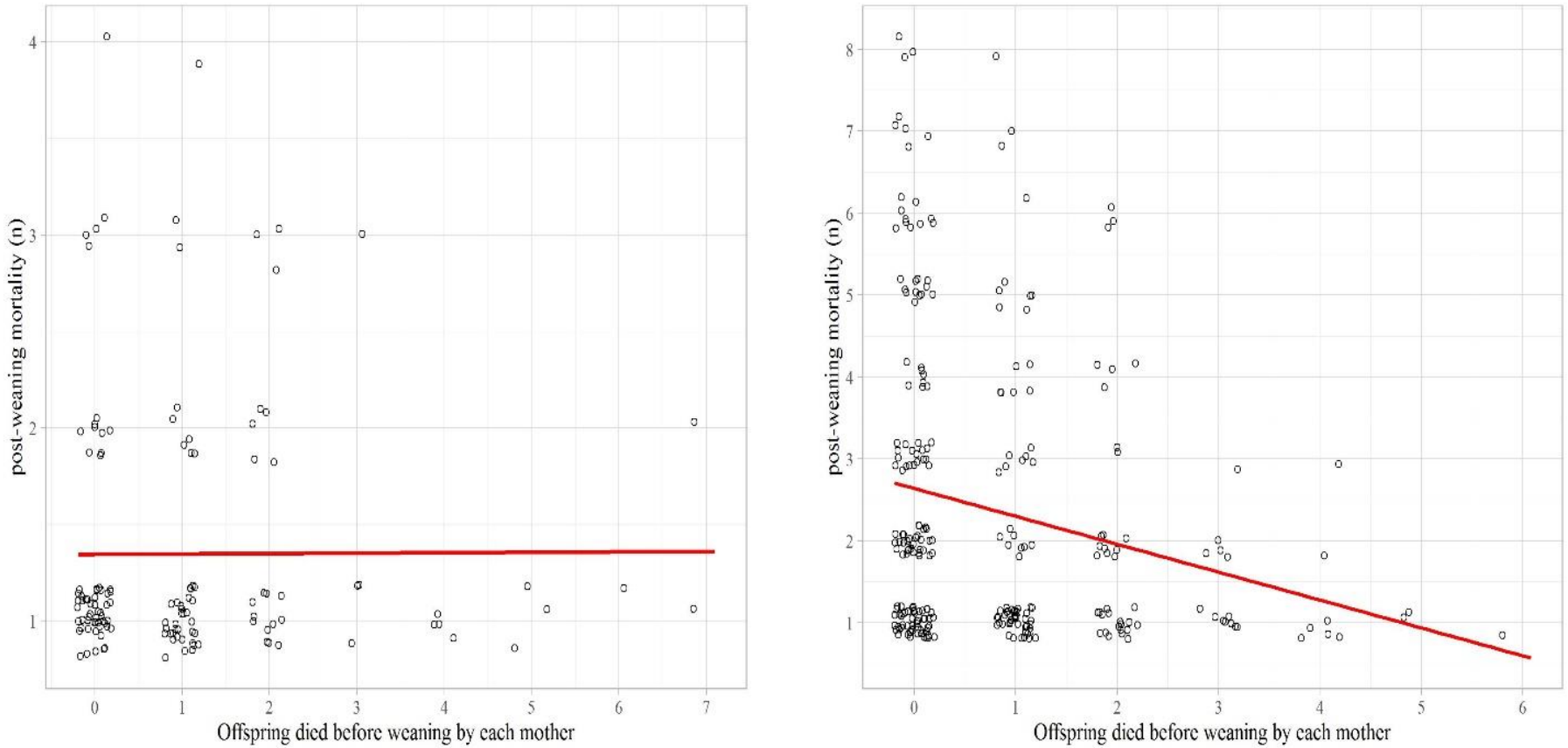


Figure 5.5.7. Linear regression for the number of offspring dead in the post-weaning period on the number of offspring dead during lactation. Left panel: before the entrance of the pathogenic *E. coli* strain *O103*. Right panel: after the entrance of this bacteria in the farm. The points have been jittered close the real one for helping the density visualization.

However, this separated analysis (before and after 2008) shows that a conclusion on the influence of these variables on the post-weaning mortality based on the linear regression approach is limited and should be take carefully. The presence of the *E. coli* influenced the results and thus these relations should be studied in a context where this pathogen is not present. Therefore, even if we described some significant relationships in the initial analysis, we cannot identify to which degree the number of offspring milked, weaned or the mortality during lactation influence the post-weaning mortality. However, the influence of few mothers in the total amount of mortality, in particular when rabbits suffered a digestive disorder, is robust.

6. DISCUSSION

The main goal of our work was to describe how robust is the post-weaning mortality pattern observed by Quevedo et al. (2003): few mothers contributing to most of the post-weaning mortality. Although our data presented some limitations, like the fact that animals of different generations of selections where not housed in the same room and we lacked information on fine-tune lactation variable (milk production, hard faces intake, nest quality, etc.), we observed a similar post-weaning mortality pattern as Quevedo et al. (2003): 22 % of mothers were responsible of ≈ 50 % of the overall post-weaning mortality. This results demonstrate that this pattern can be considered robust, and repeatable in another rabbit population (here we used the pure breed INRA1777).

With that in mind, the second aim of this work was to identify the "bad" mothers and characterize the maternal influences on the post-weaning mortality. Some authors have described the importance of the maternal environment on the correct development and survival of the newborn offspring (Rodel et al., 2009; Lumma and Clutton-Brock, 2002). This maternal environment is well known to affect offspring mortality (Rodel et al., 2009), in semi-wild environment in which no litter's manipulations are done during lactation by farmers. The main manipulation occurring in the farm is the number of offspring assigned to each female. This technique assures that each mother milks a limited and standard number of offspring. This management disrupts the connection of the number of animals born alive to the offspring milked, then the number of rabbit born alive is not considerable as subjected to the maternal environment. Indeed, we found no differences in the number of offspring born alive in mothers with different post-weaning mortality rate.

The second parameter analyzed was the number of offspring milked. In general, conditions happening in the early development may show consequence in the health and growth during the post-weaning period (Lumma and Clutton-Brock, 2002). In particular, a reducing number of offspring milked improves the milk ingestion, bringing at weaning animals at higher weight (Rodel et al., 2009). Here we found significant differences between females with different number of offspring milked: when the rabbits were raised in small litters the post-weaning survival increased with respect to rabbits raised in bigger litter. However, we could not assess if the results were due to a better maternal environment (we lack information on the weight at 21 day) or another factor. Another important aspect

shown by Prager et al. (2010) regarding the maternal effect was the negative correlation between litter size and IgG presence in the offspring.

For what concerned the number of offspring weaned and the dead offspring in lactation, we found that increasing the number of offspring weaned, increased the post-weaning mortality. The number of dead offspring in lactation is linked to the number of offspring weaned, because increasing the number of dead offspring in lactation means a reduction on the number of offspring weaned. In fact, as expected, increasing the offspring died in lactation, decrease the post-weaning mortality. This influence could be related to the date of death of the offspring. If the offspring dies in the first days of life the impact of its death should be different if it dies few days before or at weaning. At the same time, a rabbit corpse in the nest can be a great source of bacteria affecting the nest (if not removed). Furthermore, a litter with more offspring weaned, could be subjected of a higher risk of disease, if we considered that some disease affect rabbits randomly, just having more rabbits, the probability rises.

The most influent effect we observed in our data was the *E. coli* effect, affecting the farm from 2008 (from generation 41). We found that before the *E. coli* presence, there was a weak mothers' influence in the post-weaning mortality: 33% of mothers were responsible of ≈ 50% of mortality. It is also important to note that before the entrance of *E. coli*, the main exit reason in the post-weaning period was for respiratory problem. When *E. coli* affected the farm, the degree to which the mothers influenced the post-weaning mortality changed (50 % of mortality from 22 % of females). We also observed an incredibly high post-weaning mortality rate only in 4 of the 9 generations exposed to this bacterium. The result indicates that when this bacterium is present – digestive disorders – fewer mothers contributed to the post-weaning mortality; indicating a possible role of the microbiota.

Microbiota can play an important role in the mortality as it is transmitted principally by the milking mother. Particularly in rabbits, Combes et al. (2014) observed that among rabbit offspring exposed to the hard feces -that females normally depose in their nests- had less mortality than rabbits with no feces available in it. In addition, Sekirov and Finaly (2009) explained that microbiota located in the gut contribute to improve the resistance to infection through their involvement in the development of the host immune system and provision of colonization resistance. Moreover, Sonnenburg et al. (2016) observed that animals' microbiota is principally linked to the microbiota that the animals were exposed to. In the beginning of their life, rabbit offspring are exposed to the mother's microbiota, being the

main source of bacteria to which they are exposed during lactation; although cross infestations due to farmer's manipulation of the litter may occur. It is also known that the gut's microbiota is principally developed in the lactation period as reported by Abecia et al. (2007).

At the end, we also analyzed if mothers with different number of dead offspring had different number of offspring milked, weaned and dead in lactation before and after *E. coli* presence in the farm. Before the presence of bacteria there were no differences in the post-weaning mortality between females with different number of offspring milked, weaned, and died in lactation. After *E. coli* strain, the results changed, and there were no significant differences for the number of offspring dead in the post–weaning period in function of the number of offspring milked. However, mothers with different number of animals weaned and offspring dead in pre-weaning, shown a different number of dead offspring in the post-weaning period.

From these works and the fact that we show few mothers were responsible of high mortality, principally for intestinal disease, it rose our suggestion that rabbits' microbiota could be an important factor influencing the post-weaning mortality, and being the microbiota transferred from mothers to the offspring they raise, another question appears: if there is a small amount of females contributing to the overall post-weaning mortality, to which extend the microbiota of these females differs from the microbiota of females contributing to very few (or none) offspring to the post-weaning mortality? Although this question is not directly treated in the present study, the analysis here performed confirm that the identification of "good" and "bad" mothers are possible.

7. CONCLUSION

This study has produced some good results for the continuity of a bigger project, whose aim is to understand if the mother's microbiota plays an important role in the high post weaning mortality shown by few mothers. Having a data set collected for 12 years, with animals farmed in the presence and absence of *E. coli*, it was possible to know how the influence change when the rabbits are exposed to the bacteria. From this study, it was concluded that few mothers are responsible of high mortality, and this relationship is marked when the bacteria is present.

The pattern was repeatable in the different rooms used, but great differences between the period before and after the *E. coli* exposure were found. High mortality rate for digestive disorders occurred only after the bacteria colonisation.

In conclusion, for these reasons, it can be concluded that the mother influence is highly linked to the presence of digestive disorders.

8. PERSPECTIVES

The Project: Can we distinguish between “good” and “bad” mothers with respect to their influence in the post-weaning survival of their offspring? This study showed that it is possible. The next step is thus to identify if the gut microbiota of these two groups of females are different in terms of their population abundance and variation. To do so a long-term experiment (8 parturitions) is being performed. Hard feces samples of all females are being collected at first insemination and at parturitions 1, 4, 7 and 8. These samples are stored and the post weaning mortality of the litters of each single female is being followed. At the end of the sixth reproductive cycle, when all the growing rabbits are sold, the distinction between the “good” and the “bad” mothers of this rabbit population will be performed. Then their gut microbiota (those bacteria populations present in the hard feces) will be analyzed and compared. The hypothesis is that their gut microbiota differs, especially if the post weaning mortality is due to digestive disorders. If the hypothesis is confirmed, the gut microbiota assessed by the hard feces can be used as a phenotype to select good mothers and improve the health of young rabbits and avoid the use of antibiotics; a problem of public health.

One problem that researches at INRA may face with this project is the low mortality that they have in this project (now in the parturition 4) even in the absence of antibiotics in feeds used for growing rabbits.

9. REFERENCES

1. Abecia, L., Fondevila, M., Balcells, J, McEwan, N. R., 2007: The effect of lactating rabbit does on the development of the caecal microbial community in the pups they nurture. Journal of Applied Microbiology ISSN 1364-5072.

2. Berg, R.D. (1996) The indigenous gastrointestinal microflora. Trends Microbiol 4, 430–435.

3. Carabaño, R., García, J., De Blas, C., 2002. Nitrogen digestion and digestive disorders at weaning. A review. pp 42-45. In Proc. Joint Scientific Meeting WG1 (Reproduction) and WG4 (Nutrition) – COST Action 848 & ECVAN, Ispra (Varese), Italy.

4. Carabaño, R., García, J., Nicodemus, N., de Blas, C.; 2005: Nutrizione e funzione barriera intestinale nel coniglio da carne; Departamento de Producción Animal - Universidad Politécnica de Madrid; Giornate di Coniglicoltura ASIC 2005

5. Carabaño, R., Badiola, I., Chamorro, S., García, J., García–Ruiz, A.I., García–Rebollar, P., Gómez– Conde,M.S., Gutiérrez, I., Nicodemus, N., Villamide,M.J., De Blas, C. (2008). Review. New trends in rabbit feeding: influence of nutrition on intestinal health. Spanish Journal of Agricultural Research 6, 15-25.

6. Carabaño, R., Villamide, M.J., García, J., Nicodemus, N., Llorente, A., Chamorro, S., Menoyo, D., García–Rebollar, P., García–Ruiz, A.I., De Blas, C. (2009). New concepts and objectives for protein – amino acid nutrition in rabbits. Review. World Rabbit Science 17, 1–14.

7. Combes, S., Michelland, R. J., Monteils, V., Cauquil, L., Soulie, V., Tran, N. U., Gidenne, T., and Fortun-Lamothe, L., 2011: Postnatal development of the rabbit caecal microbiota composition and activity. FEMS Microbiol Ecol 77 (2011) 680–689

8. Combes, S., Gidenne, T., Cauquil, L., Bouchez, O. and Fortun-Lamothe, L., 2014: Coprophagous behavior of rabbit pups affects implantation of cecal microbiota and health status. J. Anim. Sci. 2014.92:652–665 doi:10.2527/jas2013-6394

9. Coureaud, G., R. Charra, F. Datiche, C. Sinding, T. Thomas-Danguin, S. Languille, B. Hars, and B. Schaal. 2010. A pheromone to behave, a pheromone to learn: The rabbit mammary pheromone. J. Comp. Physiol. A Neuroethol. Sens. Neural Behav.

Physiol. 196:779–790.

10. Curtis, T. P., and W. T. Sloan. 2004. Prokaryotic diversity and its limits: Microbial community structure in nature and implications for microbial ecology. Curr. Opin. Microbiol. 7:221–226.

11. Dalle Zotte A., 2014. Rabbit farming for meat purposes. Animal Frontiers, 4:62-67. doi:10.2527/af.2014-0035

12. De Blas, C. and Wiseman, J. (1998) The Nutrition of the Rabbit. Wallingford, UK: CABI Publishing

13. De Blas, E., Gidenne, T. (2010). Digestion of starch and sugars. In: De Blas, C. and Wiseman, J. (Eds.) The Nutrition of the Rabbit, 2nd Edition. CABI Publishing, Wallingford Oxon, UK. pp. 19-38.

14. De Blas, C., García, J., Carabaño, R., 1999. Role of fibre in rabbit diets. A review. Ann. Zootech. 48:3-13.

15. Drummond, H., Vazquez, E., Sanchez-Colon, S., Martinez-Gomez, M., Hudson, R., 2000. Competition for Milk in the Domestic Rabbit: Survivors Benefit from Littermate Deaths. Ethology 106, 511-526

16. Fortun-Lamothe L., Boullier, S., 2009. A review on the interactions between gut microflora and digestive mucosal immunity. Possible ways to improve the health of rabbits. 1871-1413/$ - see front matter © 2006 Elsevier B.V. All rights reserved. doi: 10.1016/j.livsci.2006.09.005

17. Gallois, M., Gidenne, T., Fortun-Lamothe, L., Le Huerou-Luron, .I, Lalles, J.P., 2005. An early stimulation of solid feed intake slightly influences the morphological gut maturation in the rabbit. Repr. Nutr. Dev. 45:109-122.

18. Gidenne, T., Carabaño, R., Garcìa, J., De Blas, C. (2010). Fibre Digestion. In: De Blas, C. and Wiseman, J. (Eds.) The Nutrition of the Rabbit, 2nd Edition. CABI Publishing, Wallingford Oxon, UK. pp.66–82.

19. Gidenne, T. (1996). Nutritional and ontogenic factors affecting caecocolic digestive physiology. In: Proceedings 6th World Rabbit Congress, Toulouse, France. Vol.1, pp. 13–28.

20. Gidenne, T. (1997) Caeco-colic digestion in the growing rabbit: impact of nutritional factors and related disturbances. Livest Prod Sci 51, 73–88.

21. Gidenne, T., 2000. Recent advances in rabbit nutrition: emphasis on fibre requirements. A review**.** World Rabbit Sci. 8:23-32.

22. Gidenne, T., and F. Lebas. 2006. Feeding behavior in rabbits. In: V. Bels, editor, Feeding in domestic vertebrates. From structure to behavior. CABI publishing, Wallingford, UK. p. 179–209.

23. Gonzalez-Mariscal, G. (2001). Neuroendocrinology of maternal behavior in the rabbit. Hormones and Behavior, 40, 125–132.

24. Gonzalez-Mariscal, G., Melo, A. I., Jimenez, P., Beyer, C., & Rosenblatt, J. S. (1996). Estradiol, progesterone, and prolactin regulate maternal nest-building in rabbits. Journal of Neuroendocrinology, 8, 901–907.

25. Gonzalez-Mariscal, G., Melo, A. I., Parlow, A. F., Beyer, C., & Rosenblatt, J. S. (2000). Pharmacological evidence that prolactin acts from late gestation to promote maternal behaviour in rabbits. Journal of Neuroendocrinology, 12, 983–992.

26. Hardman. M. J., Hull. D., Oyesiku. J. 1970: The influence of birth weight and nutrition on postnatal growth of rabbits. Biol. Neonate 16, 306-312

27. Hoy, S., and D. Selzer. 2002. Frequency and time of nursing in wild and domestic rabbits housed outdoors in free range. World Rabbit Sci. 10:77–84.

28. Hudson, R. and Distel, H. 1982: The pattern of behavior of rabbit pups in the nest. – Behaviour 79, p. 255-271

29. Hudson, R. and Distel, H. 1983: Nipple location by newborn rabbits: behavioral evidence for pheromonal guidance. Behaviour 85,260-275

30. Hudson, R. and Distel, H. 1995: On the nature and action of the rabbit nipple-search pheromone: a review. In: Chemical Signals in Vertebrates VII (Apfelbach, R., Muller-Schwarze, D., Reuter, K. and Weiler, E. eds). Elsevier Science Ltd, Oxfor, pp (223-232)

31. Hudson R, Bilkó Á, Altbäcker V. Nursing, weaning and the development of independent feeding in the rabbit. Z Säugetierkd 1996; 61:39–48.

32. Hudson R, Trillmich F.: Sibling competition and cooperation in mammals: challenges, developments and prospects. Behav Ecol Sociobiol 2007, doi:10.1 8007/s00265-007-0417-z.

33. Hull, J., Hull, D., (1982) Behavioral thermoregulation in newborn rabbits. J Comp Physiol Psychol 96:143-147

34. Koehl, P.F. (1997) Une conduite en bande amelieure-t-elle les resultats techniques? Cuniculture 24, 67–73.

35. Kovacs, M., Szendro, Z., Milisits, G., Bota, B., Biro-Nemeth, E., et al.. Effect of nursing methods and feaces consumption on the development of the bacteriodes, lactobacillus and coliform flora in the caecum of the newborn rabbits. Reproduction Nutrition Development, EDP Sciences, 2006, 46 (2), pp.205-210

36. Lebas, F., Laplace, J.P., 1972. Mensurations viscérales chez le lapin. I. Croissance du foie, des reins et des divers segments intestinaux entre 3 et 11 semaines d´age. Ann. Zootech. 21: 337-347.

37. Lincoln, D. W. 1974: Suckling: a time-constant in the nursing behavior of the rabbit. Physiol. Behav. 13,711-714

38. Licois, D. and Gidenne, T. (1999) L'emploi d'un regime deficienten fibres par le lapereau augmente sa sensibilite´ vis a vis d'une infection experimentale par une souche d'Escherichia coli enteropathogene. Proceedings of the Eighth Journe ´es Recherches Cunicoles, Paris (France), 9–10 June, pp. 101–104.Vet. Res. 36 - (2005) 601-613

39. Licois, D., Wyers, M., Coudert, P., (2005): Epizootic Rabbit Enteropaty: experimental transmission and clinical characterization.

40. Lumma, V., and Clutton-Brock, T.H. (2002) Early development, survival and reproduction in humans. Trends in Ecology and Evolution, 17, 141-147

41. Mackie, R.I. (1999) Developmental microbial ecology of the neonatal gastrointestinal tract. Am J Clin Nutr 69, 1035S– 1045S.

42. Manning, P. J., Ringler, D. H. & Newcomer, C. E. 1994: The Biology of the Laboratory Rabbit. Academic Press, London.

43. Martens, E. E., 2016: Fibre for the future. News & Views; vol l529, nature, 158-159

44. Melo, A. I., & Gonza´lez-Mariscal, G. (2003). Placentophagia in rabbits: Incidence across the reproductive cycle. Developmental Psychobiology, 43, 1–8.

45. Mendl M. The effects of litter size on variation in mother-offspring relationships and

behavioural and physical development in several mammalian species (principally rodents). J Zool Lond 1988; 215:15–34.

46. Padilha, M.T.S., Licois, D., Gidenne, T., Carre´, B. and Fonty, G. (1995) Relationships between microflora and caecal fermentation in rabbits before and after weaning. Reprod Nutr Dev 35, 375–386

47. Pascual J.J., Cervera C. y Fernandez-Carmona J. 2001. Effect of solid food intake before weaning on the performance of growing rabbits. Proccedings of COST action 848, GÖDÖLL_, pp48.

48. Peeters, J.E., Geeroms, R. and Orskov, F. (1988) Biotype, serotype, and pathogenicity of attaching and effacing enteropathogenic Escherichia coli strains isolated from diarrheic commercial rabbits. Infect Immune 56, 1442–1448.

49. Poigner J., Szendro Z., Levai A., Radnai I., Biro-Nemeth E., 2000. Effect of birth weight and litter size at suckling age on reproductive performance in does as adults. World rabbit science 2000, Vol 8 (3), 103-109.

50. Prager G., Stefanski V., Hudson R., Rodel H. D., 2010. Family matters: maternal and litter-size effect on immune parameters in young laratory rats. Brain, Behaviour and Immunity, Vol 24, Issue 8, Nov. 2010, p 1371-1378

51. Quevedo, F., Pascual, J.J., Blas, E., Cervera, C., 2003: Influencia de la madre sobre el crescimiento y la mortalidad de los gazapos en cebo; XXVIII Symposium de Cunicultura p115-122

52. Rodel, H. G., Prager, G., Stefanski, V., Von Holst, D., Husdon, R., 2008: Separating maternal and litter-size effects on early postnatal growth in two species of altricial small mammals. Physiology & Behavior 93 (2008) 826–834

53. Rodel H. G., Von Host D., Kraus C. (2009) Family legacies: short-and long-term fitness consequences of early-life conditions in female European rabbit. *Journal of Animal Ecology* 78: 789-797

54. Sekirov I & Finlay BB (2009) The role of the intestinal microbiota in enteric infection. J Physiol 587: 4159–4167.

55. Sonnenburg, E. D. *et al. Nature* 529, 212–215 (2016).

56. Wickham H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2009.

57. Xiccato, G., Trocino, A. (2008). Coniglicoltura. Apparato digerente. In: Romboli, I., Marzoni Fecia di Cossato, M., Schiavone, A., Zaniboni, L., Cerolini, S. (Eds.) Avicoltura e Coniglicoltura. Point Veterinaire Italie, Milano, Italy. pp. 404–411.

58. Xiccato, G., Trocino. A., Frgkiadakis, M., Majolini, D., 2007: Indagine sugli allevamenti di conigli in Veneto: risultati di gestione tecninca e stima dell'escrezione azotata. Giornata di coniglicoltura ASIC 2007

59. Xiccato, G., Trocino, A., Carraro, L., Fragkiadakis, M., Majolini, D. (2008). Digestible fibre to starch ratio and antibiotic treatment time in growing rabbits affected by epizootic rabbit enteropathy. In: Proceedings 9th World Rabbit Congress, Verona, Italy. pp. 847–851. Accessed on December 2, 2011.

60. Xiccato, G., Trocino, A., Sartori, A., Queaque, P.I., 2001. Influence de l'âge du sevrage précoce et de l'aliment sur le développement des organes digestifs et des fermentations caecales chez le lapin. pp 199-202 in Proc. 9èmes J. Rech. Cunicole, Paris, France

61. Zarrow, M. X., Denenberg, V. H., & Anderson, X. O. (1965). Rabbit: Frequency of suckling in the pup. Science, 150, 1835–1836

SITOGRAFIA

www.anci-aia.org

www.fao.org/faostat

R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

10. ACKNOWLEDGEMENTS

I would like to thank to Dr. Davi Savietto for giving me the opportunity to do my Master Thesis at the INRA of Toulouse. Thanks for helping me and teach me with patience how to work.

I would like also to thank all team in the Bat E, that help me to beyond my limits and myself, learning also new language. In a particular way, I want to thank Malo, Charlotte and Bruno that help me a lot especially at the very beginning of my experience.

Ringrazio profondamente la mia relatrice e docente, Prof.ssa Antonella Dalle Zotte, per avermi dato l'opportunità di fare questa esperienza all'estero, la quale mi ha dato l'opportunità di confrontarmi con nuove realtà, di farmi viaggiare e soprattutto crescere come persona. La ringrazio per tutto l'impegno profuso nei confronti di noi studenti e per averci spronato a sperimentare questa straordinaria esperienza. Grazie per aver ben sponsorizzato Toulouse: qui ho conosciuto una nuova realtà e imparato una nuova lingua. Grazie a questa esperienza ora credo di più in me stesso, e so di poter arrivare ovunque io voglia, sebbene il percorso non sia mai semplice.

Grazie a tutti per avermi aiutato a crescere e per avermi aiutato a raggiungere questo grande traguardo.